DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

Bioprocessing of Berry Materials with Malolactic Fermentation

NIKO MARKKINEN

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Department of Life Technologies

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Food Development (tech)

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NIKO MARKKINEN

Food Chemistry and Food Development
Department of Life Technologies

TURKU, FINLAND – 2021

Food Chemistry and Food Development
Department of Life Technologies
University of Turku, Finland

Supervised by
Professor Baoru Yang, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland

Adjunct Professor Oskar Laaksonen, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland

Reviewed by
Professor Giuseppe Spano, Ph.D.
Department of Agricultural, Food, and Environmental Sciences
University of Foggia
Foggia, Italy

Professor Georgios-Ioannis Nychas, Ph.D.
Department of Food Science and Human Nutrition
Agricultural University of Athens
Athens, Greece

Opponent
Professor Marco Gobbetti, Ph.D.
Faculty of Sciences and Technology
Free University of Bozen-Bolzano
Bolzano, Italy

Research director
Professor Baoru Yang, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland

The originality of this dissertation has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service

ISBN 978-951-29-8621-7 (print)
ISBN 978-951-29-8622-4 (pdf)
ISSN 2323-9395 (print)
ISSN 2323-9409 (pdf)
Painosalama Oy – Turku, Finland 2021

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ABSTRACT

Malolactic fermentation is used by wine industry to decrease acidity and introduce odor compounds through the metabolic activity of ethanol-tolerant lactic acid bacteria. In this work, this oenological approach was used to modify flavor chemistry of berry materials with low consumer value. The aim was to determine fermentation conditions that lead to effective deacidification and possibly to other chemical changes that would improve sensory and functional properties, and thus consumer value, of various berry materials.

For the practical work of the thesis, juices from sea buckthorn (*Hippophaë rhamnoides* L.), chokeberry (× *Sorbaronia mitschurinii*, old name *Aronia mitchurinii*) and lingonberry (*Vaccinium vitis-idaea* L.) were fermentated with multiple strains of *Lactiplantibacillus plantarum* and *Oenococcus oeni*, pre-cultivated either in a typical basal medium or in an acclimation medium. Multiple methodologies combining chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy were applied for targeted, semi-targeted, and non-targeted analysis of metabolites in the juices before and after fermentation.

While chokeberry juice was fermentable as such, fermentation of natural sea buckthorn juice was ineffective in most cases. However, increasing juice pH from natural 2.7 to 3.5 or acclimating cells prior to inoculation allowed fermentation of sea buckthorn with all studied strains. At the natural pH of sea buckthorn juice, no sugars were fermented or flavonol glycosides metabolized. At the natural pH of chokeberry juice, sugars were fermented along with malic acid, and quercetin glycosides, chlorogenic acid, and other phenolic acids present in the juice were metabolized by *L. plantarum*. All fermentations that used lingonberry as raw material failed due to the high content of benzoic acid.

During fermentation of sea buckthorn and chokeberry, the metabolism of malic acid yielded mainly lactic acid, while the metabolism of quinic acid led to the formation of protocatechuic acid, catechol, shikimic acid, and 3,4,5-trihydroxy-1-cyclohexanoic acid. Other microbe-related metabolites detected in the fermented sea buckthorn juice were acetic acid, ethanol, isovaleric acid, phenyllactic acid, succinic acid, 1,3-dihydroxyacetone, trehalose, maltose, GABA, and oxaloacetic acid. It was concluded that depending on the strain of *L. plantarum*, acetate production during the fermentation of sea buckthorn juice was supported by the production of ethanol or succinic acid, or quinic acid metabolism, to consume excess NADH.

Nearly all the identified volatile compounds from sea buckthorn juice were esters with a fruity descriptor. Fermentation with *L. plantarum* increased the content of volatile acids, i.e. acetic acid, 3-methylbutanoic acid, and free fatty acids as well as the content of buttery ketones (acetoin) and various alcohols.

While the concentration of fruity esters was decreased during fermentation, the content of benzyl alcohol (floral) and 2-undecanone (fruity) were increased. The content of fatty-acid derived aldehydes was decreased in all fermented samples.

In summary, fermenting sea buckthorn juice for a shorter period (36 h) and at a lower pH (2.7) led to an effective deacidification via malolactic fermentation without the consumption of sugars. Reducing fermentation time resulted to a decrease in formation of volatile acids and less loss of ester compounds present in the juices. On the other hand, fermentation for longer time (72 h) and at an elevated pH (3.5) led to stronger protection of ascorbic acid from oxidation, greater formation of antimicrobial compounds (3-phenyllactic acid, acetic acid, lactic acid), and greater metabolism of secondary metabolites (phenolic compound and quinic acid metabolism). These results can be applied to the product development of novel fermented beverages utilizing acidic berry or fruit material rich in malic acid as raw material.

SUOMENKIELINEN ABSTRAKTI

Viiniteollisuus hyödyntää laajasti viinien happamuuden vähentämiseksi ja aromin muokkaamiseksi malolaktista fermentaatiota, jonka taustalla ovat etanolia sietävät maitohappobakteerit. Tässä työssä tätä viiniteollisuuden lähestysmistapaa hyödynnettiin haastavanmakuisten marjojen maku- ja aromikemian muokkaamiseen. Työn tavoitteena oli selvittää ne fermentaatio-olosuhteet, joissa marjojen happamuuden vähentäminen ja muut aistittavan laadun kannalta positiiviset muutokset kemiallisessa koostumuksessa saadaan aikaan mahdollisimman tehokkaasti.

Väitöskirjan kokeellisessa osassa tutkittiin soveltuvatko tyrnimarjasta (*Hippophaë rhamnoides* L.), marja-aroniasta (× *Sorbaronia mitschurinii*, vanha nimi *Aronia mitchurinii*), ja puolukasta (*Vaccinium vitis-idae* L.) valmistetut mehut raaka-aineeksi malolaktiseen fermentaatioon. Mikrobeina käytettiin useita eri kantoja lajeista *Lactiplantibacillus plantarum* ja *Oenococcus oeni*. Mehut valmistettiin joko ilman tai entsyymikäsittelyn avustamana. Solukasvatukset tuotettiin tyypillisessä elatusaineessa tai sopeutusliuoksessa. Työssä hyödynnettiin laajasti erilaisia analyyttisiä työkaluja, joihin lukeutuvat erilaiset kromatografiset menetelmät, massaspektrometria, ja ydinmagneettinen resonanssispektroskopia. Kohdennettuilla, osin kohdennettuilla ja kohdentamattomilla analyyseillä määritettiin muutokset fermentaation kannalta olennaisten aineenvaihduntatuotteiden koostumuksessa.

Mikrobit pystyivät fermentoimaan aroniamehua sellaisenaan, mutta muokkamattoman tyrnimehun fermentaatio onnistui vain vaihtelevasti. Tyrnimehun pH:n nosto 2.7:stä 3.5:een ja solujen sopeutus happamiin olosuhteisiin ennen fermentaatiota mahdollisivat onnistuneen fermentaation kaikilla testikannoilla. Kun tyrnimehua fermentoitiin ilman pH:n säätöä, mikrobit eivät metaboloineet sokereita tai tyrnimehun flavonoliglykosideja. Sen sijaan *L. plantarum* metaboloi aroniamehun sokereita, kversetiini glykosideja, klorogeniinihappoa ja fenolisia happoja myös mehun luontaisessa pH:ssa. Puolukan fermentaatio epäonnistui kaikissa testiolosuhteissa, mikä johtui puolukkamehun korkeasta bentsoehappopitoisuudesta.

Omenahapon aineenvaihdunta tuotti pääasiassa maitohappoa sekä aronia- että tyrnimehussa, kun taas kviinihapon metabolia tuotti protokatekiinihappoa, sikimihihappoa ja 3,4,5-trihydroksi-1-sykloheksaanihappoa. Muita mikrobiperäisiä metaboliitteja, joita fermentoidusta tyrnimehusta tunnistettiin, olivat etikkahappo, etanoli, isovaleriaanahappo, fenyylimaitohappo, meripihkahappo, dihydroksiasetoni, trehaloosi, maltoosi, γ-aminovoihappo, ja oksaloetikkahappo. Tuloksista selvisi, että kannasta riippuen tyrnimehun fermentaation aikana *L. plantarum* tuotti etanolia tai meripihkahappoa, tai

metaboloi kviinihappoa, kuluttamaan ylimääräisen NADH-kofaktorin, jota muodostui asetaatin tuottamisesta.

Valtaosa sekä määrällisesti että koostumuksellisesti tyrnimehusta tunnistetuista haihtuvista yhdisteistä olivat estereitä, joilla on hedelmäinen ominaishaju. Fermentaatio lisäsi haihtuvien happojen määrää, joita olivat tässä tapauksessa etikkahappo ja erilaiset vapaat rasvahapot. Tämän lisäksi fermentaatiossa vapautui asetoiinia, jolla on voimainen ominaishaju. Vaikka hedelmäisten estereiden määrä laski fermentaation aikana, bentsyylialkoholin (kukkainen ominaishaju) ja 2-undekanonin (hedelmäinen ominaishaju) konsentraatiot kasvoivat. Rasvahappoperäisten aldehydien määrät laskivat kaikissa fermentoiduissa mehuissa.

Tuloksista voitiin päätellä, että tyrnimehun fermentointi lyhyemmän ajan (36 t) ja pH:ta muokkaamatta (aloitus-pH 2.7) happamuutta voitiin vähentää tehokkaasti ilman sokerien fermentointia. Lyhyellä fermentaatioajalla oli myös se etu, että tyrnin hedelmäisten esterien määrä tippui vähemmän, ja mehuun ei muodustunut yhtä paljon haihtuvia happoja verrattuna pitkään fermentaatioaikaan (72 t). Kun mehun pH oli korkeampi fermentaation aloitusvaiheessa (3.5) askorbiinihappo hapettui vähemmän fermentaation aikana ja mehuun muodostui enemmän antimikrobisia yhdisteitä (3-fenyylimaitohappo, etikkahappo, maitohappo). Tämän lisäksi korkeammassa pH:ssa mikrobit metaboloivat enemmän sekundäärimetaboliitteja (fenoliset yhdisteet, kviinihappo). Tämän väitöskirjan tuloksia voidaan hyödyntää uudenlaisten fermentoitujen juomien kehittämiseen, joiden raaka-aineena käytetään paljon omenahappoa sisältäviä marja- tai hedelmämateriaaleja.

LIST OF ABBREVIATIONS

3-MB-3MB	3-methylbutyl 3-methylbutanoate
ACPY	2-Acetyl-1-pyrroline
ACTPY	2-Acetyltetrahydropyridine
ArAA	Aromatic amino acid
BA	Benzoic acid
BcAA	Branched-chain amino acid
CFU	Colony forming units
COOH	Carboxyl group
CPFA	Cyclo-propane fatty acid
Di-HBA	Dihydroxybenzoic acid
DMDS	Dimethyl disulfide
DMTS	Dimethyl trisulfide
DP	Degree of polymerization
DW	Dry weight
E-2MB	Ethyl 2-methylbutanoate
E-3MB	Ethyl 3-methylbutanoate
EC	Epicatechin
EGCG	Epigallocatechin
EGCG	Epigallocatechin gallate
E-HA	Ethyl hexanoate
EMP	Embden–Meyerhof–Parnas
E-OA	Ethyl octanoate
EtOH	Ethanol
ETPY	2-Ethyltetrahydropyridine
E-β-G	Ethyl β-D-glucopyranoside
FID	Flame ionization detector
FOS	Fructooligosaccharides
FW	Fresh weight
GA	Gallic acid
GABA	γ-aminobutyric acid
GOS	Galactooligosaccharides
HBA	Hydroxybenzoic acid
HCA	Hydroxycinnamic acid
HMBA	4-methylthio-2-hydroxybutanoate
hsp	Heat-shock protein
ILA	Indole lactic acid
KMBA	4-methylthio-2-ketobutanoate
MLF	Malolactic fermentation
MRS	De Man, Rogosa and Sharpe

OH-PLA	Hydroxyphenyllactic acid
PAC	Proanthocyanidins
p-CA	p-Coumaric acid
PCA	Principal component analysis
PDA	Photodiode-array detector
PDC	p-Coumaric acid decarboxylase
PEP	Phosphoenolpyruvate
Pho	Phosphate
PLA	Phenyllactic acid
PTS	Phosphotransferase system
SB	Sea buckthorn
SFA	Saturated fatty acids
TCA	Tricarboxylic acid
USFA	Unsaturated fatty acids
VOC	Volatile compounds
VP	Vinylphenol
Ref	Reference

LIST OF ORIGINAL PUBLICATIONS

I. Markkinen, N.; Laaksonen, O.; Nahku, R.; Kuldjärv, R.; Yang, B. Impact of lactic acid fermentation on acids, sugars, and phenolic compounds in black chokeberry and sea buckthorn juices. *Food Chem.* **2019,** 286, 204–15.

II. Markkinen, N.; Laaksonen, O.; Yang, B. Impact of malolactic fermentation with *Lactobacillus plantarum* on volatile compounds of sea buckthorn juice. *Eur. Food Res. Technol.* **2021**, 247(3), 719–36.

III. Markkinen, N.; Pariyani, R.; Jokioja, J.; Kortesniemi, M.; Laaksonen, O.; Yang, B. NMR-based metabolomics approach on optimization of malolactic fermentation of sea buckthorn juice with *Lactiplantibacillus plantarum. Food Chem.* **2022**, 366, 130630.

1 INTRODUCTION

Fermentation does not require invention by humans. It is a process that occurs naturally in organic material due to the metabolic activity of bacteria and fungi. Early humans had no access to our modern understanding of hygiene, biochemistry, and microbiology, but presumably by trial and error, they learned how to preserve milk and vegetables in a manner that would generate fermented foods palatable and safe to consume. Their approach was probably a spontaneous fermentation by controlling the temperature or salinity and using a "starter culture" from a previous successful batch. Nevertheless, fermentation was used in a systematic manner and on a large scale as early as 4000 BCE by the Egyptians to produce bread and by the Babylonians to produce beer [1].

In addition to allowing storage of perishable materials for extended periods, fermentation, as a low temperature method, allowed preservation of compounds sensitive to heat or oxidation. Thus, foods such as sauerkraut provided a source of vitamin C during seasons when fresh fruits and vegetables were not available. For the same reason, fermented foods were also significant in the long seafaring expeditions during 18th century as they helped to avoid the development of scurvy within the crew. Nevertheless, it was not until 1857 that Lois Pasteur discovered that micro-organisms were the cause of fermentation and the spoilage of foods [1].

Many traditional fermented foods such as kimchi, soy sauce, and kombucha are still produced today either with classical spontaneous fermentation or with the help of modern science and research in a highly controlled, hygienic environment using micro-organisms developed specifically for a selected purpose. The reason why fermentation is still a relevant processing method today is primarily because that for the production of many products there is no alternative. Wines, cheeses, sourdough bread, and traditional salami can only be produced with fermentation [2].

However, studies related to fermenting fruit materials for other purposes than to alcoholic beverages is a rather recent approach. Most of the research on this topic have only been published within the last five to ten years. One possible explanation is the current global consumer trend related to products that promote health and well-being, and fermented foods are often associated with health-promoting properties [3].

While the aim of traditional fermentation was to preserve milk or vegetables, the fermentation of fruits and berries can be used to improve not only microbiological but also oxidative stability, as the fermentation of plant materials often leads to increased antioxidant capacity [4]. Another approach is to modify the flavor of fruit materials. Lactic acid bacteria can deliver beneficial impact on odor though the biosynthesis of compounds that complement fruity or floral

characteristics of the raw material [5]. Malolactic fermentation (MLF) can be utilized for deacidication of materials with intense sourness [6] while phenolic and flavonoid modification is of interest from both a health and flavor point-of-view [7]. This approach has been adopted from the wine industry, as wines can undergo spontaneous secondary fermentation where ethanol-resistant lactic acid bacteria present in the wine ferment residual L-malic acid to D/L-lactic acid, modify phenolic compounds, and generate aromatic ketones, alcohols, and esters to further develop the flavor of the wine [8]. If probiotic strains are used for fermentation, then the microbe itself can deliver a further health benefit to the consumer as an addition to the fermented food [9].

This doctoral thesis work focused on berry materials that have been described as sour, bitter, and/or astringent, including lingonberry, sea buckthorn, and chokeberry. All of these berry species have well-established health benefits [10–12], but low consumer value due to their unpleasant sensory properties [13–15]. The approach selected was to focus primarily on the MLF for deacidification purposes, and secondarily to the modification of phenolic and other odor compounds. The goal was to investigate whether *L. plantarum* can ferment the berry materials, and to measure strain-dependent differences in the modification of the berry flavor chemistry. The impact of initial pH, the contents of the basal medium, and fermentation time, were also studied to determine the optimal fermentation conditions. Both volatile and non-volatile flavor compounds were studied to capture the complete picture of changes in chemical composition during fermentation. Connections between fermentation factors and chemical composition was established using various uni- and multivariate statistical methods. Moreover, whenever possible, connections between the observed changes were related to the known metabolic pathways of *L. plantarum*. The goal was to provide a systematic approach to flavor modification by binding together the substrate-product connection, and to discuss why the specific gene expressed in that setting. Therefore, the intention of the literature review of this dissertation was not only to report qualitative and quantative changes observed in fermented food models but also to establish the metabolic systems of *L. plantarum* relevant to the fermentation of plant-based food materials.

2 REVIEW OF THE LITERATURE

2.1 General features and taxonomy of *Lactiplantibacillus plantarum*

Lactiplantibacillus plantarum (former name *Lactobacillus plantarum*) is a versatile species of lactic acid bacteria that is encountered in a variety of environmental niches. It is especially common in spontaneously fermented plant foods, where it is important for finishing the fermentation. Such foods include sauerkraut, kimchi, table olives, and sourdough bread. Due to its high alcohol tolerance, the species is also common in the MLF of wines. Furthermore, *L. plantarum* isolates have been detected in dairy and meat fermentations and are also among the species able to colonize the human GI tract, with some strains possessing probiotic properties [9,16,17]. In addition, some *L. plantarum* strains are able to improve the bioavailability of micronutrients such as iron [18], or are able to degrade organophosphorus insecticides [19].

In 2003, the complete genome of *L. plantarum* WCFS1 isolated from human saliva was sequenced [20]. Although Lactobacilli, in general, have a small genome [9], *L. plantarum* has one of the largest genomes in lactic acid bacteria, about 3.3 Mbp [20]. The G+C content of the chromosome is 44.5%. Based on the metabolism of carbohydrates, the species has been defined as facultative heterofermentive [20]. By accumulating large intracellular pool of Mn^{2+}, the species is able to tolerate oxidative stress, and is thus defined as facultative anaerobe [20].

The large genome of *L. plantarum* leads to the ability to utilize a large collection of carbon sources and to tolerate stress from acidity, alcohol, salinity, and phenolic compounds, among other things. These two characteristics together explain why *L. plantarum* can adapt to a large variety of environments. Fermentation with *L. plantarum* has the potential to improve sensory, nutritional and functional properties of the food matrix [21], while the ability to acidify the raw material and to produce antifungal acids and antimicrobial plantaricins improve shelf-life [9,22,23].

Lactobacillus plantarum was initially named by Orle-Jensen (1919). Currently, two subspecies of *L. plantarum* have been identified and a number of closely taxonomical species (**Fig. 1**). In 1987, *Lactobacillus pentosus* (type strain 124-2 (=DSM 20314)) was identified as a separate species from *L. plantarum* [24]. The key difference was the ability of *L. pentosus* to ferment D-xylose and glycerol. However, this difference is not unequivocal, as there are strains of *L. plantarum* that can metabolize glycerol [25]. In 1996, a separate species *Lactobacillus paraplantarum* (type strain CST 10961 (=DSM 10667)) was identified [26]. The main difference was the ability of *L. paraplantarum* to ferment

methyl α-D-glucose, while the majority of *L. plantarum* strains have no gene for the metabolosis of the compound [25].

Later, a separate subspecies, *L. plantarum* subsp. *argentoratensis* was identified using phylogenetic analysis; the type strain assigned was DKO 22T (= DSM 16365). Other strains not belonging to this subspecies were renamed *L. plantarum* subsp. *plantarum* [27].

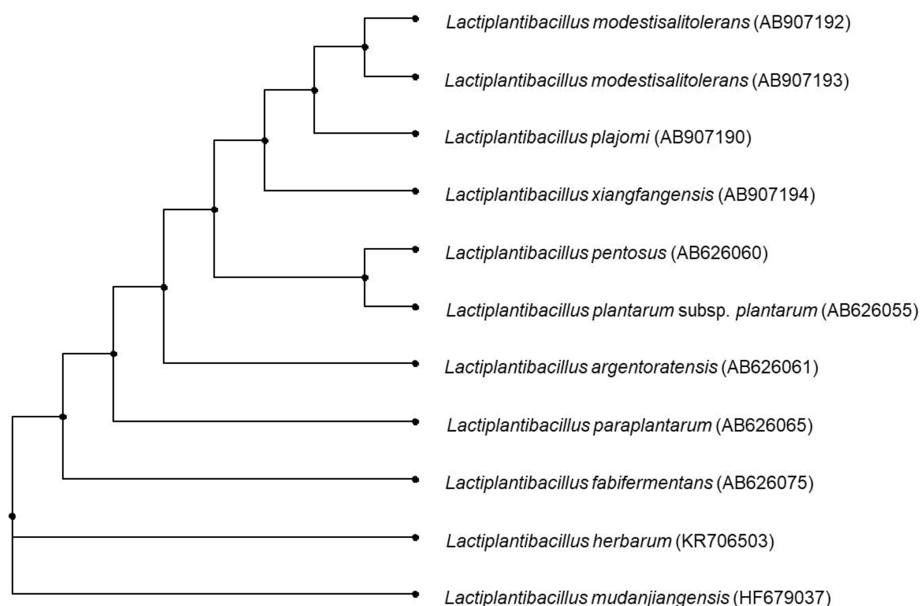


Fig. 1. Phylogenic tree of *L. plantarum* and the so-called *L. plantarum* family of related species. Accession number in parentheses. Generated with Phylogeny Server (https://ggdc.dsmz.de/).

Later, two additional novel *Lactobacillus* species with an over 98 % 16S rRNA sequence similarity to *L. plantarum* were detected, *Lactobacillus* LMG 24284 which was isolated from cocoa bean fermentations in Ghana [28], and another novel strain which was isolated from traditional Chinese pickles [29]. However, more detailed genomic analyses indicated that they were novel species, which were then named *Lactobacillus fabifermentans* [28] and *Lactobacillus xiangfangensis* [29], respectively. Other species that have so far been identified and suggested to belong to the *L. plantarum* group are *L. herbarum*, *L. plajomi*, *L. modestisalitolerans,* and *L. mudanjiangensis* [30].

In 2020, a proposal for reclassification of the genus *Lactobacillus* into 25 distinct genera based on a polyphasic approach was published [31], in which *Lactobacillus plantarum* subsp. *plantarum* and *Lactobacillus plantarum* subsp. *argentoratensis* were renamed as *Lactiplantibacillus plantarum* and *Lactiplantibacillus argentoratensis*, respectively. For the sake of clarity, the

abbreviation *L. plantarum* according to the previous nomenclature is used throughout the thesis for both before-mentioned species.

2.2 Carbohydrate metabolism of *L. plantarum*

Glycolysis is the main form of energy production under anaerobic conditions in many lactic acid bacteria, including *L. plantarum*. The importance of glycolysis is emphasized by the fact the presence of glucose downregulates both amino acid metabolism [32] and β-glucosidase activity [33].

2.2.1 Carbohydrates transport systems

L. plantarum cells uptake carbohydrates either through carbohydrate specific transporters or through multiple sugar ABC transporters [20]. Genomic analyses of *L. plantarum* WCFS1 has revealed 25 complete PTS enzyme complexes [20]. In the strain *L. plantarum* NCU116, a strain-specific fructose/mannose-inducible PTS complex has been identified [34]. The PTSs are a class of bacterial sugar uptake systems that utilize energy from phosphoenolpyruvate (PEP) to phosphorylate sugars upon transfer inside the cell. PEP, in turn, is derived from the glycolysis pathway. Once inside the cell, sugars are metabolized to produce ATP and to maintain NAD^+/NADH homeostasis. Additionally, compounds derived from sugar catabolism are precursors for many important components required for growth, including pyridine, purine and histidine biosynthesis from ribulose-5-phopshate, and aromatic amino acid biosynthesis from PEP *via* shikimate pathway [34,35].

2.2.2 Homo- and heterolactic fermentation

Typical classification of lactic acid bacteria is based on their glucose/sugar metabolism. The two major pathways are homolactic and heterolactic fermentation, which produce lactic acid alone, or lactic acid, acetate and ethanol, respectively [36]. Genomic studies have revealed that *L. plantarum* possess the genes for both homo- and heterolactic fermentation [20,34,37]. Therefore, *L. plantarum* is effectively a facultative heterofermentative species.

In a homofermentative pathway (**Fig. 2**), glucose is metabolized to pyruvate. One mole of glucoses produces two moles of pyruvate. Pyruvate is then reduced with NADH to either L-lactate or D-lactate by L-lactate dehydrogenase or D-lactate dehydrogenase, respectively. The two isomers are produced in equimolar amounts [20]. Taking into account the sugar transport, the EMP pathway consumes two ATP, while producing 4 ATP, leading to a net production of 2 ATP. Regarding redox potential, NADH regenerated in the oxidation of glyceraldehyde-3-phosphate is converted to NAD^+ when pyruvate is reduced to

lactate, and thus NAD^+/NADH homeostasis is maintained in the homofermentative pathway. The presence of multiple copies of lactate dehydrogenase genes suggests the preference for pyruvate reduction into lactate in *L. plantarum*. However, pyruvate can also be converted into acetyl-CoA by pyruvate dehydrogenase and dihydrolipoamide dehydrogenase. Acetolactate synthase on the other hand can catalyze the formation of α-acetolactate from pyruvate [34].

In the heterofermentative pathway, hexoses are first fermented into xylulose-5-phosphate and ultimately into lactate, acetate, or ethanol through pentose phosphate pathway. Acetyl-phosphate is formed through phosphoketolase pathway, with either ethanol (from acetyl-CoA) or acetate as the final product. Acetyl-CoA is also used for fatty acid synthesis [38].

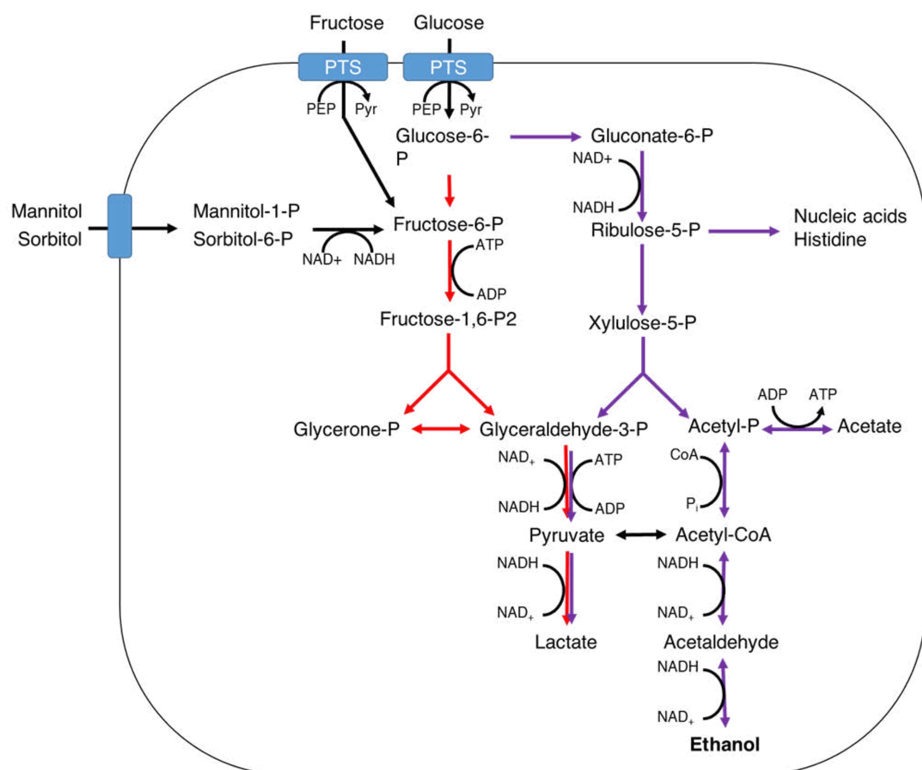


Fig. 2. The homofermentative Embden–Meyerhof–Parnas (EMP) pathway (red arrows) and the heterofermentative pentose phosphate pathway (purple arrows) in addition to sugar alcohol utilization identified from *L. plantarum*. Adapted from [39–42].

2.2.3 Sugar alcohol fermentation

The sugar alcohols sorbitol and mannitol are transported to cell through D-ribose and sorbitol PTS, and this is present in most *L. plantarum* strains and closely

related species (*L. pentosus* and *L. paraplantarum*). After transfer into the cell, these sugar alcohols are fermented to pyruvate [39]. When sugar alcohol fermentation was studied in strain *L. plantarum* NF92, in the early growth stages lactic acid was the main end-product. However, in the late growth stages, ethanol production was more pronounced as mannitol and sorbitol induced expression of aldehyde-alcohol dehydrogenase-encoding gene (*adhE*). Furthermore, it was observed that the expression was mediated through two DNA-binding regulators, AcrR (activator) and Rex (repressor).

Excess NADH accumulation in the early growth stages from mannitol and sorbitol utilization removes Rex from its binding site, allowing expression of *adhE*, suggesting that ethanol production is activated to maintain NAD^+/NADH homeostasis [39]. Interestingly, AcrR was also a key expression factor for the ethanol tolerance of *L. plantarum* [43]. Therefore, it is seems that in *L. plantarum* AcrR activates pathways related to ethanol production as well as mechanisms to protect the cell from the toxic effects of the ethanol.

2.2.4 Di-, tri-, oligo- and polysaccharide metabolism

L. plantarum can thrive in a variety of plant materials due to its ability to catabolize various more complex carbohydrates into monosaccharides (**Table 1**). *L. plantarum* is able to degrade lactose *via* β-glucosidase while maltose and trehalose are fermented by α-glucosidase [44]. Sucrose, on the other hand, is metabolized by β-fructosidase, encoded by gene *sacB* [45]. Glucosidases are also important enzymes in the modification of phenolic compounds (**Section 2.5.3.1**) and volatile precursors (**Section 2.9**).

Genomic analysis of strain *L. plantarum* NCU116 revealed genes for α-amylase, neopullanase, and α-glucan branching enzyme. As this strain was extracted from an environment rich in fermentable poly- and oligosaccharides (Chinese sauerkraut), it is plausible that the ability to ferment complex carbohydrates is beneficial for adaptation to this environmental niche [34]. Degradation of complex carbohydrates improves digestibility of foods by removing fermentable di-, tri- and oligosaccharides that could otherwise cause gastrointestinal discomfort [46].

2.3 Organic acid metabolism of *L. plantarum*

2.3.1 Citrate and tartarate metabolism

The first step of citrate metabolism is transport into the cell by an *L. plantarum* citrate transporter (CitP, *lp_1022*). The transporter protein has been registered as a divalent anion:Na^+ symporter [41], meaning that the CitP uptakes deprotonated citrate with the concomitant uptake of a sodium ion [47].

Table 1. Genes and the corresponding enzymes detected from *L. plantarum* associated with the metabolism of complex carbohydrates (adapted from [44]).

Gene	***Enzyme***	***Function***
dexB	α-Glucosidase	Activity on dextran; hydrolyzes α-(1→6)-glucosidic linkages, isomaltooligosaccharides and panose but not maltose
glgP	Glycogen phosphorylase	Activity on maltotriose, higher maltodextrins, and glycogen but not on maltose
LacG	Phosho-β-galactosidase	Lactose ➔ Glucose + Galactose-Pho
LacL	β-Galactosidase	GOS hydrolysis
LacM	β-Galactosidase	GOS hydrolysis
lacS	Lactose permease	Di- and trigalactooligosaccharides transporter
LacZ	β-Galactosidase	GOS hydrolysis
malH	6-Phospho-α-glucosidase	Maltose ➔ D-Glucose + D-Glucose-6-Pho
malL	Amylopullulanase	Hydrolyzes α-(1→6)-glucosidic linkages in pullulan and amylopectin, also hydrolyzes α-(1→4)-glucosidic linkages in polysaccharides
malN	Amylopullulanase	Same as malL
malP	Maltose phospholyrase	Maltose ➔ D-Glu + β-D-Glucose-1-Pho
melA	α-Galactosidase	Hydrolyzes terminal α-D-galactose residues in α-D-galactosides
pgmB	β-Phosphoglucomutase	β-D-Glucose-1-Pho ➔ β-D-Glucose-6-Pho
Pts1BCA	Sucrose phosphotransferase	Transports FOS into the cytosol while transferring a phosphoryl-moiety on to the glucose residue of the FOS
sacA	Fructosidase	Same function as bfrA
sacK1	Fructokinase	D-Fructose ➔ D-Fructose-6-Pho

Abbreviations: Pho, phosphate; GOS, galactooligosaccharides; FOS, fructooligosaccharides.

Next, citrate is converted into oxaloacetate and acetate. The reaction is catalyzed by citrate lyase, which consists of γ, β and α subunits encoded by *citD*, *citE*, and *citF*, respectively [48]. In *L. plantarum* WCFS1, citrate lyase regulator (*citR*) and citrate lyase genes form a gene cluster *citR-mae-citCDEF* showing associated expression with malate dehydrogenase (*mae*) [20].

Oxaloacetate is a substrate for various enzymes. One pathway is a decarboxylation of oxaloacetate to pyruvate *via* the Mae enzyme. Citrate fermentation can therefore in theory be utilized for energy production, as pyruvate can be converted to acetyl-CoA through pyruvate dehydrogenase complex, and subsequently to acetate, yielding 1 mole ATP per 1 mol of citrate. An *oad* gene, encoding an oxaloacetate decarboxylase was also identified in *L. plantarum* NCU116 [34]; this enzyme produces a Na^+ gradient which could be used for cellular functions related to cytoplasmic membrane [49].

In lactic acid bacteria, a significant portion of the metabolic flux of pyruvate is its reduction into lactate. However, in materials with an initially low pH or acidified by fermentation (due to release of lactate from glycolysis), pyruvate can be converted to neutral compounds acetoin, diacetyl and 2,3-butanediol [50,51] in order to maintain internal pH homeostasis (**Fig. 3**). All three previously mentioned compounds contribute to the buttery and caramel notes [52]. While pyruvate can be generated from various pathways and substrates, in wine MLF, accumulation of diacetyl and acetoin is associated with citrate metabolism. Therefore, the presence of citrate lyase genes is often screened from strains intended for wine MLF [53,54]. In *L. plantarum*, the optimal pH range for citrate degradation and subsequent acetoin formation in synthetic wine medium was between pH 4–5. In addition, the presence of glucose inhibited acetoin formation from citrate while the presence of fructose induced it [48].

In wines fermented with *O. oeni* diacetyl is the main component produced in the fermentation that contributes to the buttery notes [55]. However, since *L. plantarum* lacks genes for the diacetyl reductase (*butA*) and the 2,3-butanediol dehydrogenase (*butC*) to produce diacetyl and 2,3-butanediol, respectively, the main end-product is acetoin (**Fig. 3**) [34,37]. In food fermentations with *L. plantarum*, small amounts of diacetyl have been detected, most likely due non-enzymatic oxidation of α-acetolactate. Diacetyl has a sensory threshold value of 0.2–2.8 mg/L, while acetoin and 2,3-butanediol have a sensory threshold of 150 mg/L and 600 mg/L, respectively [55]. Therefore, it can be speculated that *L. plantarum* is a poor contributor to buttery notes compared to *O. oeni* in MLF.

L. plantarum contains genes for a partial tricarboxylic acid cycle [56], which is relevant for citrate and malate metabolism (**Fig. 3**). Through this pathway, oxaloacetate can be reduced to malate, and further dehydrated and reduced to fumarate and succinate, respectively. *L. plantarum* have been reported to utilize this reductive pathway (citrate-to-succinate) to regenerate NAD^+ in carrot juice

fermentation [35] while the pathway was down-regulated under salt stress [57]. In addition, citrate metabolism was activated under ethanol toxicity [58]. The missing pathways in the TCA cycle explains several amino acid auxotrophies of *L. plantarum* (see **Section 2.8**).

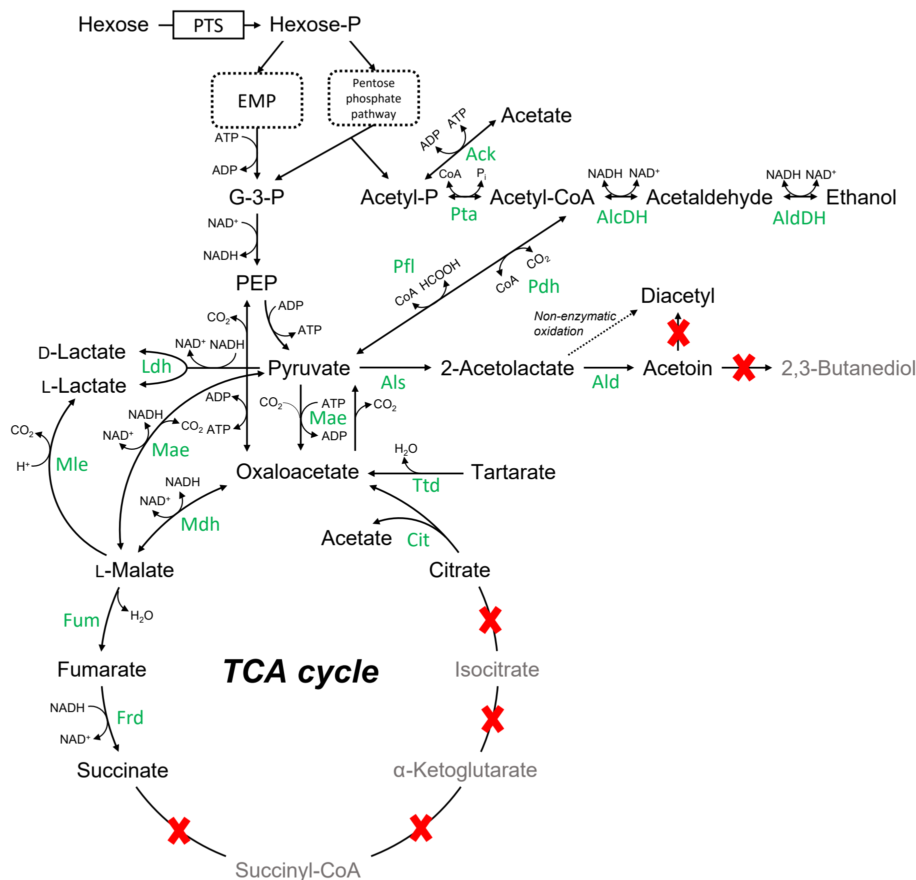


Fig. 3. Citrate, malate and tartarate metabolism pathways in *L. plantarum*, in addition to pathways that lead to acetate and acetoin formation. Genes associated with the pathways are marked in green color. Pathways missing in *L. plantarum* are marked with a red cross, and thus the metabolites *L. plantarum* is unable to produce have gray font. Adapted from [34,37,40–42,56]. For details of the enzymes and genes, see **Supplementary Table S1**.

While citrate metabolism by wine bacteria and *L. plantarum* has been extensively studied, there is very little research on tartarate metabolism in *L. plantarum*. Nevertheless, genes for tartarate utilization have been identified from *L. plantarum* WCSF1 [20]. Tartarate shares the same entry point to citrate in

metabolism, as L(+)-tartaric acid is converted to oxaloacetate by tartarate dehydrases (*ttdAB*) (UniProt accession numbers F9UMQ6 and F9UMQ5, respectively). Tartarate is transported into the cytoplasm via tartarate transport protein (*ttdP*) (accession number: F9UMQ3).

2.3.2 Quinic acid metabolism

Quinic acid metabolism in *L. plantarum* was first established in the 1970s by Whiting and Coggings [59–61] (**Fig. 4**). It was discovered that quinic acid can be metabolized through two separate pathways, in both of which 3-dehydroshikimic acid is the intermediate. Dehydration of 3-dehydroshikimic acid ultimately yields catechol, referred to as the *oxidative pathway* as the NADH generated upstream is not consumed. The second pathway yields 3,4-dihydroxy-cyclohexane-1-carboxylic acid as the end-product. As the pathway generates a surplus of $NAD(P)^+$, it has been referred to as the *reductive pathway*. Enzymes and genes related to this pathway are yet to be characterized.

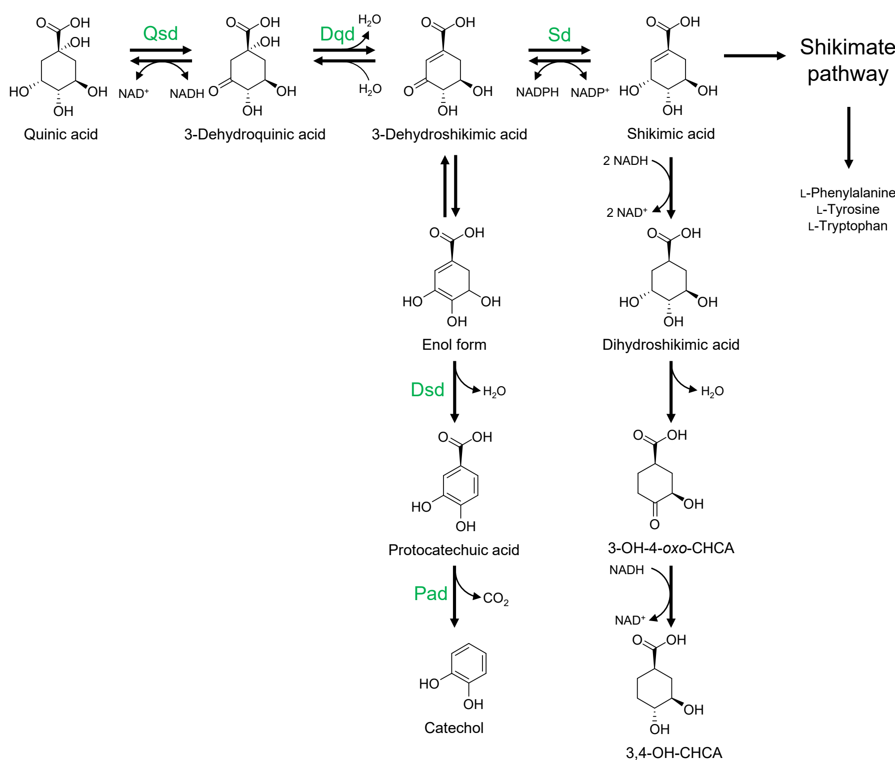


Fig. 4. Quinic acid metabolism in *L. plantarum*. Adapted from [35,60]. For details of the enzymes (green font) and genes, see **Supplementary Table S1**.

While quinic acid metabolism yields no energy, alternating metabolic flux between the two pathways allows *L. plantarum* to regenerate redox cofactors. In

heterofermentive bacteria the ability to utilize alternative electron acceptors, such as hydroxycinnamic acids, allows increased ATP production from the conversion of acetyl phosphate to acetate [62]. Additionally, shikimic acid produced in the metabolism of quinic acid can also serve as precursor for the biosynthesis of aromatic amino acids [35].

2.3.3 Malolactic fermentation (MLF)

L-Malic acid is transported into cell through C4-dicarboxylate permease (*mleP123*), which is induced by L-malate. In *L. plantarum*, unlike in many other malolactic bacteria, *mleP* is not downregulated by glucose [63]. MLF is regulated by the MleR [64].

In *L. plantarum*, MLF can occur through three separate pathways. First pathway is reduction and decarboxylation of malate to pyruvate *via* malate oxaloacetate-decarboxylating malate dehydrogenase (*mae,* MAE pathway), often referred as the 'malic enzyme'. Pyruvate can in turn be converted to D- or L-lactate. However, similar to citrate metabolism, pyruvate can be utilized for ATP production through mixed acid fermentation [37]. As the name suggests, the same enzyme also converts oxaloacetate to pyruvate, and is therefore also relevant in citrate metabolism.

The second pathway, same as previously mentioned, is the conversion of malate to pyruvate, but through a different mechanism. Here malate is first reduced to oxaloacetate by malolactic dehydrogenase (encoded by *mdh*), followed by decarboxylation to pyruvate. While this activity is typically associated with the heterofermentative *Lactobacillus* species, such as *L. fermentum*, some but not all *L. plantarum* strains also possess a gene for this pathway [34,37]. The third pathway is a direct decarboxylation of L-malate to L-lactate in a Mn^{2+}- and NAD^{+}-dependent reaction by so-called 'malolactic enzyme', encoded by *mleS* in *L. plantarum* (referred here-on-out as the MLE pathway) (**Fig. 3** and **Fig. 5**). The exact catalytic mechanism of this single-step decarboxylation of the malolactic enzyme remains to be elucidated.

Earlier it was thought that MLE produces no ATP as there is a lack of substrate level phosphorylation but is rather utilized for de-acidification of the growth medium [65]. However, in *O. oeni* the MLE system was downregulated in H^{+}-ATPase deficient mutants, suggesting that in *O. oeni* MLF is primarily associated with metabolic energy production [66].

In *L. plantarum* an electrochemical system for ATP production *via* the MLE system has been suggested earlier (**Fig. 5**) [63]. At an optimal pH range for MLF (pH 3.5–4.5) [63] the majority of L-malic acid is in monoanionic form (pK_{a1} = 3.40, pK_{a2} = 5.20). At high malate concentrations, malate(−1) (MAL^{-}) enters the cell through diffusion by a malate transporter (MleP). Next, MleS decarboxylates the non-α-hydroxy acid group of the malate. Being the carboxylic group that is

predominately disassociated at the cytoplasmic pH of *L. plantarum*, a proton is consumed in the process. As lactic acid has pK_a of 3.86, at cytoplasmic pH the end-product is predominately lactate(−1). Efflux of negatively charged lactate(−1) forms the basis of an electrochemical gradient (i.e., proton motive force), which in turn enables ATP production by H^+-ATPase. In this way metabolic energy is produced while maintaining intracellular pH [63].

Typically, the MLE system is presented with MAL^- as the main substrate. However, due to higher pH in the cytosol compared to the extracellular space, MAL^- is, to a large extent, further disassociated to a malate(−2) (MAL^{2-}) and a proton. Recently, it was observed that for the *O. oeni* malolactic enzyme, MAL^{2-} is the preferred substate, followed by MAL^- and L-malic acid, respectively [67]. However, no such investigations exist regarding the MleS of *L. plantarum*. The end-product of MleS is lactate(−1) when the substrate is MAL^{2-} and lactic acid when the substrate is MAL^- or malic acid. If energy production from MLE is as Olsen et al. [63] suggested, then it makes no difference whether the substrate is MAL^- or MAL^{2-}, since the proton released from disassociation of lactic acid or MAL^-, respectively, is consumed by MleS during decarboxylation. Therefore, a more significant role is played by the transportation of L-malic acid (MleP), and only the intake of deprotonated forms of L-malic acid would allow energy production. In this context, at a very low pH (<3.0), where protonated L-malic acid is the predominant form, in theory, the system would become electroneutral and would no longer produce ATP. On the other hand, at a higher pH the MLE system becomes less effective as well, potentially due to reduced ability to intake L-malate.

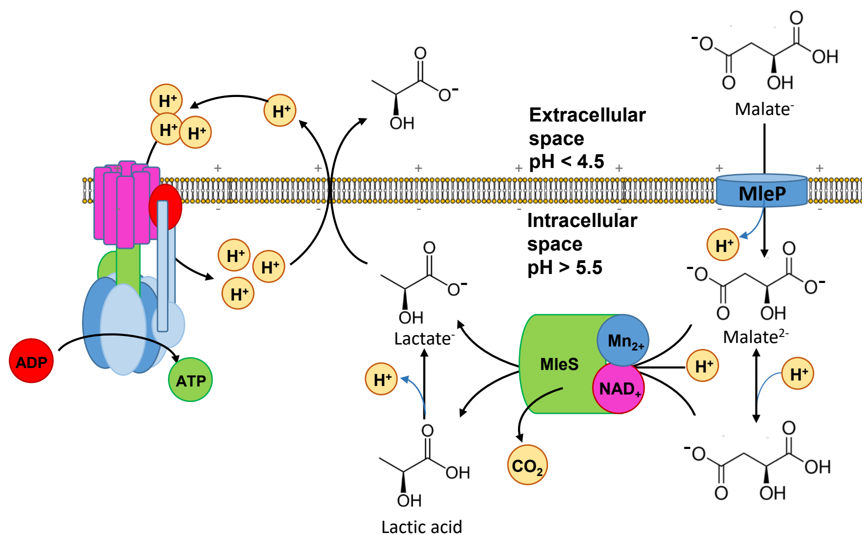


Fig. 5. Putative electrochemical mechanism of metabolic energy generation from the MLE pathway in *L. plantarum* at a high L-malic acid concentration (> 5 mM) [63]. MleP, L-malate transporter; MleS, malolactic enzyme of *L. plantarum*.

The ATP generating mechanism varies between different bacterial species. In *L. lactis*, MLE utilizes an anion exchange symport, i.e. the intake of malate with a simultaneous efflux of lactate [68]. In some species MLE pathways exists but contributes little to ATP production. For example, in *Lactobacillus casei* inactivation of the MLE pathway increased growth rate by increasing the metabolic flux to the MAE pathway. This suggests that in *L. casei* the MLE pathway is utilized for de-acidification [69].

2.4 Volatile and phenolic ester metabolism in *L. plantarum*

2.4.1 Classification of esterases and lipases

This section focuses on a discussion of the relevant genes and enzymes present in *L. plantarum* responsible for hydrolysis and the formation of esters. Small volatile esters are important class of odor compounds in wines, fruits, and berries [70], and thus enzymes that affect these compounds are relevant for flavor modification during fermentation. Non-volatile phenolic compounds, such as phenolic acids, flavonols and tannins, are responsible not only for the bitter and astringent characteristics of wines and plant materials but also for the health properties, which are often connected through ester bonds to sugars, alkyl chains and other acyl groups. Such compounds include chlorogenic acid, an ester of caffeic acid and quinic acid. Therefore, esterases able to hydrolyze phenolic compounds, such as tannases, are important in fermentation of plant materials for the modification of functional and flavor properties.

Esterases can be divided to several classes based on the substrate specificity. Carboxylesterases hydrolyze water-soluble and short-to-medium-length aliphatic esters, while arylesterases hydrolyze aromatic esters. Lipases, on the other hand, tend to show high activity with long-chain, fat-soluble esters. Both esterases and lipases belong to the α/β hydrolase enzyme superfamily with shared catalytic mechanisms for both ester hydrolysis and formation [71].

2.4.2 Acyl transferases and reverse esterases

Various reverse esterases and acyl transferases of fermenting yeasts are important drivers for wine and beer flavor [72]. Chemical analyses have shown that malolactic bacteria *O. oeni* and *L. plantarum* can facilitate the formation of esters, including the formation of ethyl lactate or diethyl succinate [73,74]. However, no specific enzymes have been characterized from *L. plantarum* with acyl transferase or reverse esterase activities. Cell-free extracts of *O. oeni* showed reverse esterase activity by forming ethyl esters of octanoic, butanoic, and

hexanoic acids. In the same study, cell-free extracts of *L. plantarum* produced moderate amounts of ethyl hexanoate. [75]

2.4.3 Carboxylesterases, arylesterases, and lipases

Genomic studies have revealed various esterases from *L. plantarum* (**Table 2**). These include carboxylesterases (Cest-2923, Lp_2631), arylesterases (Lp_1002), lipases (Lp_3562, Lp_1760), and feryol esterases/tannases (Est_1092, TanA, TanB). In general, esterases and lipases of *L. plantarum* prefer acetates and butyrates as substrates (**Fig. 6**). Arylesterases and carboxylesterases prefer shorter alkyl chains, while lipases retain higher activity with more lipophilic esters, especially Lp_1760. Indeed, Lp_1760 is among the few lipases of *L. plantarum* with high activity on tributyrin. While this is typically relevant in dairy and especially in cheese fermentation, in plant materials with high lipid content, such as sea buckthorn or avocado, this lipase might become significant.

Esterases with the potential to hydrolyze small water-soluble aliphatic esters identified in *L. plantarum* include Lp_0796, LpEst1 and Lp_1002. Apart from isobutyl acetate, Lp_1002 had activity with all aliphatic esters within the test library, showing wide substrate specificity (**Fig. 6**). In addition, Lp_1002 retains its activity better compared to other esterases at acidic conditions (**Fig. 6**). Therefore, arylesterase Lp_1002 has characteristics that emphasize its relevance in the MLF of wine or fruit materials with high acidity. However, it is not clear if *lp_1002* is a common gene within species and strains belonging to the genus *Lactiplantibacillus*.

2.4.4 Feryol esterases and tannases

Relevant to the hydrolysis of hydroxycinnamic acid esters is the fact that gene *lp_0796* encodes a feruloyl esterase commonly present in *L. plantarum* [76]. Furthermore, an uncommon feruloyl esterase/tannase gene *est_1092* was detected in seven strains of *L. plantarum* out of the 28 tested. Esterase Est_1092 was capable not only of hydrolyzing all the tested HCA esters, but also all the tested hydroxybenzoic esters and gallotannins, showing a very broad range of hydrolytic activity with various phenolic esters (**Fig. 7**). While the *lp_0796* expression level was not affected by methyl ferulate or methyl gallate, expression of *est_1092* was induced by the former and inhibited by the latter [77]. Est_1092 also maintains a relatively high activity in acidic conditions, meaning that it retains activity even at low pH fermentations (**Fig. 6**). While *L. plantarum* have shown the ability to hydrolyze caffeyolquinic acids (i.e., chlorogenic acids), no enzyme that is able to hydrolyze the compound effectively has been detected.

Table 2. Esterase, tannase, and lipase genes identified from *L. plantarum*.

Gene	***Type of enzyme***	***Presence in L. plantarum***	***Inducers***	***Inhibitors***	***Ref.***
Cest-2923	Carboxylesterase				78
est_1092	Feryol esterase/tannase	In 7/28 strains	Gene expression: methyl ferulate (HCA)	Gene expression: methyl gallate (HBA)	77
lp_0796	Feryol esterase	In all tested strains			76,79
lp_1002	Arylesterase		Enzyme activity: $MnCl_2$, $CaCl_2$, EtOH (4%), citric acid (0.5-1 g/L)	Enzyme activity: Malic acid (>5 g/L)	80
lp_1760	Tributyrin esterase		Enzyme activity: $CaCl_2$, $MgCl_2$, NaCl (<10%)	Enzyme activity: NaCl (>15%), $CaCl_2$ (>10%), Lactic acid (>1g/L)	81
lp_2631	Carboxylesterase		Enzyme activity: $MnCl_2$, malic acid (>5 g/L), citric acid (0.5 g/L)	Enzyme activity: EtOH (>8%), lactic acid (> 5 g/L), citric acid (>5 g/L), tartaric acid (>5 g/L)	71
lp_3562	Halotolerant lipase		Enzyme activity: $MgCl_2$, $MnCl_2$, $NaCl_2$		82
$tanA_{Lp}$	Extracellular tannase	In 4/28 strains	Enzyme activity: $CaCl_2$		83
$tanB_{Lp}$	Intracellular tannase	In all tested strains	Gene expression: methyl gallate Enzyme activity: KCl, $CaCl_2$		84–86

Abbreviations: HCA, hydroxycinnamic acid; HBA, hydroxybenzoic acid.

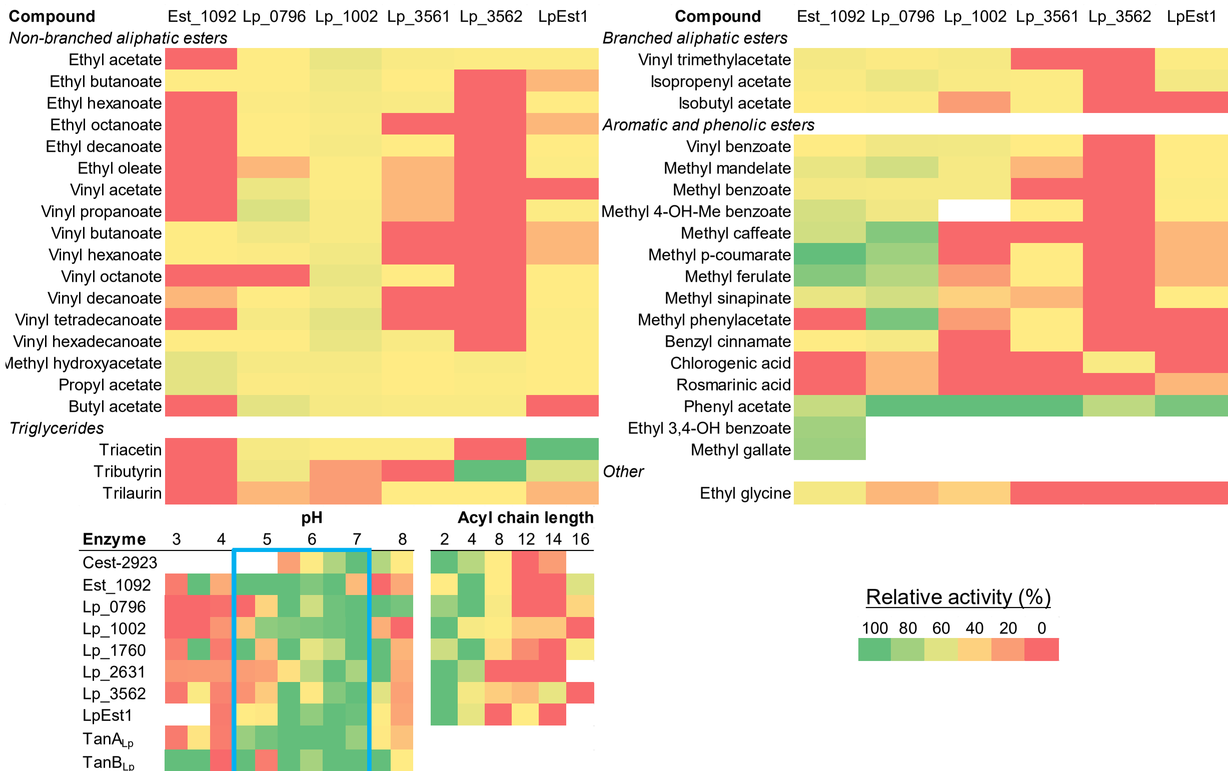


Fig. 6. Heatmap of the relative activity of *L. plantarum* esterases (Cest-2923, Lp_0796, Lp_1002, Lp_1760, Lp_2631, LpEst1), tannases (Est_1092, $TanA_{Lp}$, $TanB_{Lp}$) and lipase (Lp_3562) with compounds in the general ester library and chromogenic substrates (*p*-nitrophenol esters) in addition to the relative activity in pH from 3 to 8. Blue box indicates expected intracellular pH range in fermentation of acidic plant material, assuming that growth is halted when intracellular pH reaches 4.5 [87]. For references and additional details, see **Table 2.**

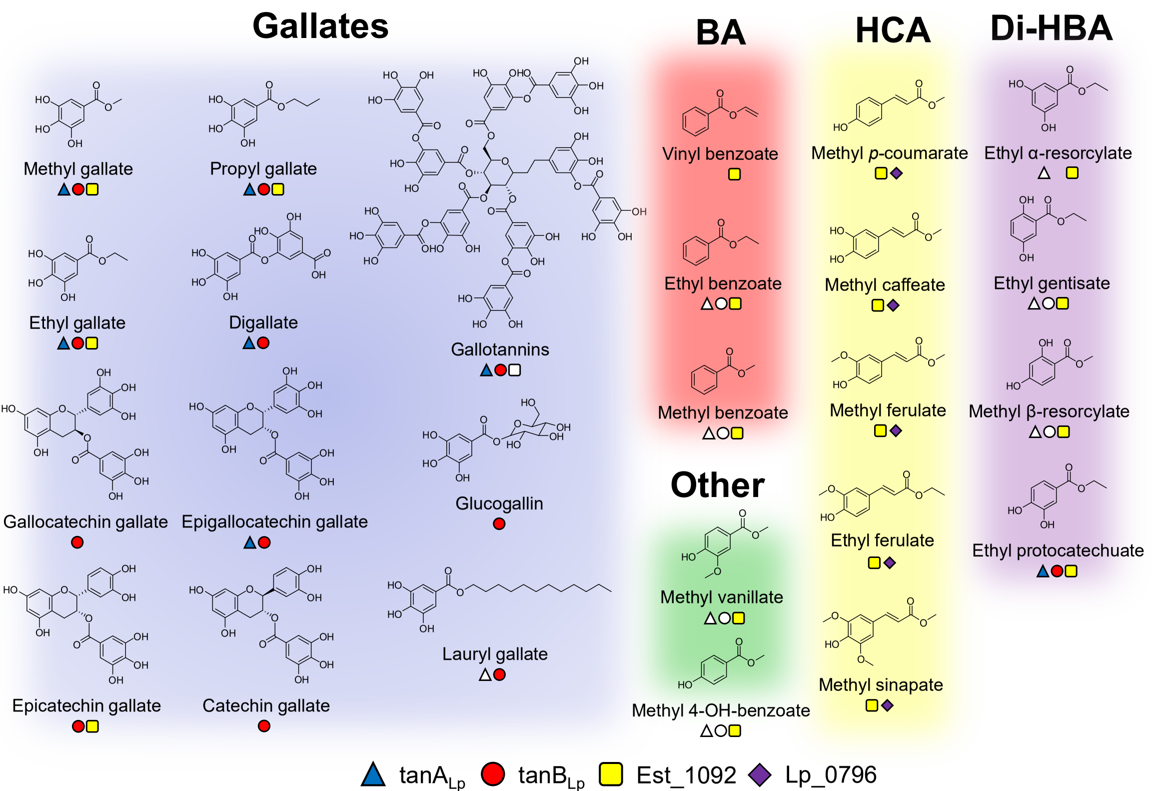


Fig. 7. Hydroxybenzoic and hydroxycinnamic acid esters that are substrates of the esterases and tannases identified from *L. plantarum*. A symbol with a white filling means that the compound was not a substrate for the corresponding enzyme while missing symbol means that no report exists for the enzyme-substrate combination. For the references and additional details, see **Table 2.**

Besides Est_1092, two tannase genes *tanBLp* (also known as *tanLp1* or *lp_2956*) and *tanALp* have been identified in *L. plantarum*. tanBLp hydrolysed only substrates with at least two phenolic groups.

Moreover, the esterified COOH group must be on oxidized benzene ring, and must not be in an *ortho* position to one of the OH rings (**Fig. 7**). In accordance with this, chlorogenic acid, ellagic acid, quercetin, catechin, epicatechin, epigallocatechin were resistant to tanLp1 [84]. While *tanBLp* is commonly present in *L. plantarum* and in related species (such as *L. paraplantarum* and *L. pentosus*) [85], *tanALp* was detected only in certain strains of *L. plantarum* [83]. Additionally, *tanBLp* was inducible by methyl gallate, while expression of *tanALp* was not affected by the presence of a substrate. Moreover, *tanALp* was detected to be an extracellular enzyme while *tanBLp* was considered to be an intracellular enzyme [83–85].

While both Est_1092 and $tanB_{Lp}$ possess ability to hydrolyze gallic acid and protocatechuic acid esters, due to differences in the expression pattern in the presence of methyl gallate, and the gene being common in *L. plantarum*, it is likely that *tanBLp* is among the relevant genes of *L. plantarum* responsible for metabolism of hydroxybenzoic acid esters.

2.5 Phenolic acid metabolism in *L. plantarum*

2.5.1 Hydroxybenzoic acid metabolism

The main enzyme in *L. plantarum* that detoxifies hydroxybenzoic acids has been identified as thr gallate decarboxylase encoded by *lpdBCD* (**Fig. 8**). LpcC was identified as the catalytic unit, while the role of LpdB is to generate prenylated flavin mononucleotide cofactor for LpcC. The role of LpdD has not been established. Expression of *lpdBCD* was inducible by its substrate, gallic acid [88]. The gallate decarboxylase of *L. plantarum* had activity with only gallic acid and protocatechuic acid, yielding pyrogallol and catechol, respectively [88,89]. This suggests that that OH-group in both *para*- and *meta*-positions are required for the LpdC. For example, *p*-hydroxybenzoic acid was not metabolized by *L. plantarum* CECT 748T [89]. This is in accordance with *L. plantarum* esterase and tannase activities, which have shown preference with gallates and protocatechuates (**Fig. 7**).

Interestingly, gene for the catalytic unit *lpdC* and gene encoding inducible tannase *tanBLp*, which catalyzes hydrolysis of gallotannins, are only 6.5 kB distant from each other, which suggested concomitant activity [90]. This was confirmed when a more in-depth understanding of the transcriptomic response of *L. plantarum* to gallate exposure was determined by [90]. It was observed that if gallic acid content is substantial enough, gallic acid that enters the *L. plantarum*

cell is bound to the TanR protein. This complex in turn binds to the DNA and works as a transcriptional factor to induce expression of *lpdBCD*, *tanR*, *gacP* (gene for gallate and pyrogallol transport protein), and *tanBLp* to effectively convert gallate to pyrogallol and subsequently remove the latter from the intracellular space [90].

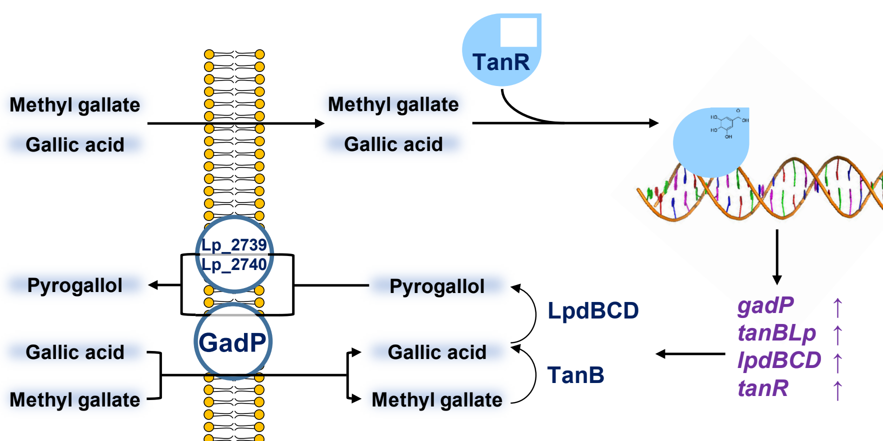
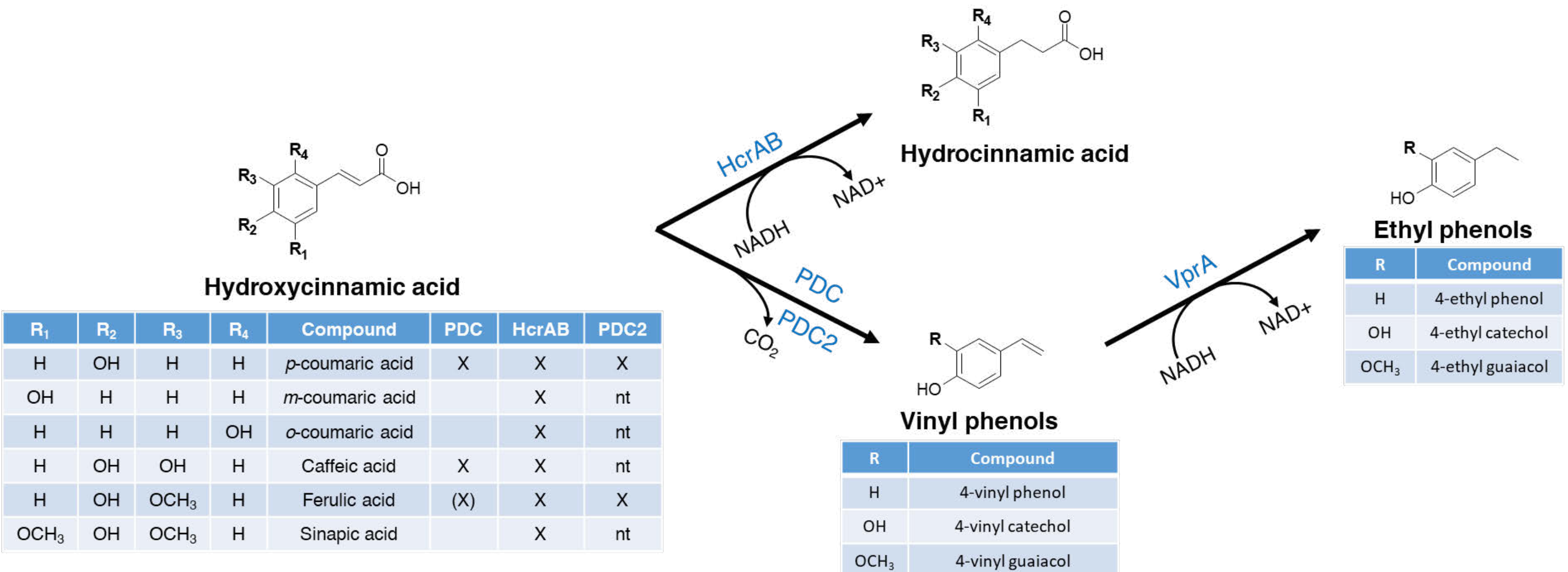


Fig. 8. Genomic response to the gallate exposure in *L. plantarum* to counter hydroxybenzoic acid toxicity. Adapted from [88,90]. For details of the enzymes and genes, see **Supplementary Table S1**.

2.5.2 Hydroxycinnamic acid metabolism

Hydroxycinnamic acids (HCA) are a class of phenolic acids with 3-phenylprop-2-enoic acid backbone combined with at least one hydroxyl group in the aromatic ring, commonly found in fruits, vegetables, coffee, and tea [91]. *L. plantarum* can metabolize several HCA either by decarboxylation or by reduction of the side-chain double bond (**Fig. 9**).

The most defined decarboxylase of HCAs in *L. plantarum* is the *p*-coumaric acid decarboxylase (*pdc*, *lp_3665* or LpPDC) [92]. As the name suggests, the main substrate is *p*-coumaric acid (*p*-CA). The *pdc* gene is inducible by *p*-CA [93,94]. Many HCAs, including *p*-CA, are toxic to *L. plantarum* due to the disturbance to the lipid bilayer [95]. Therefore, it can be speculated that the high toxicity of *p*-CA to *L. plantarum* has created evolutionary pressure to generate effective detoxification pathways. Besides *p*-CA, LpPDC has showed activity equal to *p*-CA with caffeic acid. However, depending on the conditions, LpPDC has little or no activity with ferulic acid [92,94]. Additionally, *m*- and *o*-coumaric acids are not substrates for this enzyme [92]. Therefore, it has been concluded that the hydroxyl group in the *para*-position is necessary for LpPDC. On the other hand, reduced hydroxycinnamic acids (e.g. phloretic acid) are not substrates of LpPDC, indicating that the double bond in the side chain is also relevant for the activity [89].



Hydroxycinnamic acid

R_1	R_2	R_3	R_4	Compound	PDC	HcrAB	PDC2
H	OH	H	H	*p*-coumaric acid	X	X	X
OH	H	H	H	*m*-coumaric acid		X	nt
H	H	H	OH	*o*-coumaric acid		X	nt
H	OH	OH	H	Caffeic acid	X	X	nt
H	OH	OCH_3	H	Ferulic acid	(X)	X	X
OCH_3	OH	OCH_3	H	Sinapic acid		X	nt

Vinyl phenols

R	Compound
H	4-vinyl phenol
OH	4-vinyl catechol
OCH_3	4-vinyl guaiacol

Ethyl phenols

R	Compound
H	4-ethyl phenol
OH	4-ethyl catechol
OCH_3	4-ethyl guaiacol

Fig. 9. Confirmed pathways of hydroxycinnamic metabolism in *L. plantarum* and associated enzymes with potential substrates (adapted from [92,94,96,97]). Abbreviation "nt" means "not tested". For details of the enzymes and genes, see **Supplementary Table S1**.

Since the knockout mutant of the *pdc* gene still weakly decarboxylated *p*-CA and ferulic acid, it was reported that another HCA decarboxylase system besides LpPDC exists in *L. plantarum*. Additionally, this system was induced better by ferulic acid than *p*-CA.[98]

Besides decarboxylation, the double bond in the side chain of HCA and vinyl phenols can be reduced by the enzymes HcrAB and VprA, respectively. HcrAB, compared to LpPDC, has a wider number of identified substrates [96]. HCA reductase activity is not commonly present in lactic acid bacteria; however, it seems to be a common trait among *L. plantarum*. While phenolic acid decarboxylases are important to detoxify phenolic acids, the suggested main function of phenolic reductases is to regenerate NAD^+ [96,97]. In optimal growth conditions (i.e., in MRS medium), the main metabolites of *p*-CA and caffeic acid by strain *L. plantarum* TMW 1.460 were vinyl phenol and vinyl catechol, respectively, while the main metabolite of ferulic acid was dihydroferulic acid [95].

Vinyl and ethyl phenols are volatile compounds, and in addition, 4-vinyl guaiacol, 4-ethyl guaiacol, 4-vinyl phenol, and 4-ethyl phenol are also aroma-active [52]. While volatile phenols are often considered off-odors and a sign of *Brettanomyces* spoilage in wines [97], 4-vinyl guaiacol is an important compound in certain wheat beers to generate “clove”-like aroma [99].

2.5.3 Other metabolic routes of phenolic modification

2.5.3.1 Glycosidases

In berries and fruit, the majority of phenolic compounds such as flavonols and flavan-3-ols are naturally present as glycosides [100]. Therefore, these compounds are potential targets for enzymes related to carbohydrate metabolism, especially glycosidases (**Fig. 10**).

When glycosidic activity was compared among 20 strains of *L. plantarum*, all the tested strains were able to release β-D-glucose, α-D-glucose, and β-D-galactose from *p*-nitrophenol, while lacking activity on glycosides with β-D-fucose, β-d-xylose and β-D-rhamnose as the sugar moiety. The activity was associated with aryl glycosidase *lp_3629*. Besides *p*-nitrophenols, β-D-glycosidic bond of esculin, phloridzin, and quercetin-glycoside were hydrolysed [101]. While no activity on β-D-rhamnoside was detected in the previous study, genes encoding two α-rhamnosidases (*rhaB1* and *rhaB2*) have been identified from *L. plantarum* [102]. Both enzymes showed preference to the α-1,6-linkage of L-rhamnose to β-D-glucose. Therefore, phenolics with rutinose as sugar moiety are potential targets. It was found that rutin (quercetin-rutinoside) and hesperidin (hespertin-rutinoside) were most affected [102]. Rutin is among the most common flavonol glycosides in fruits and berries [100], and therefore, depending on substrate availability, this enzyme potentially has great significance in flavonol transformation of plant material when fermented with *L. plantarum*.

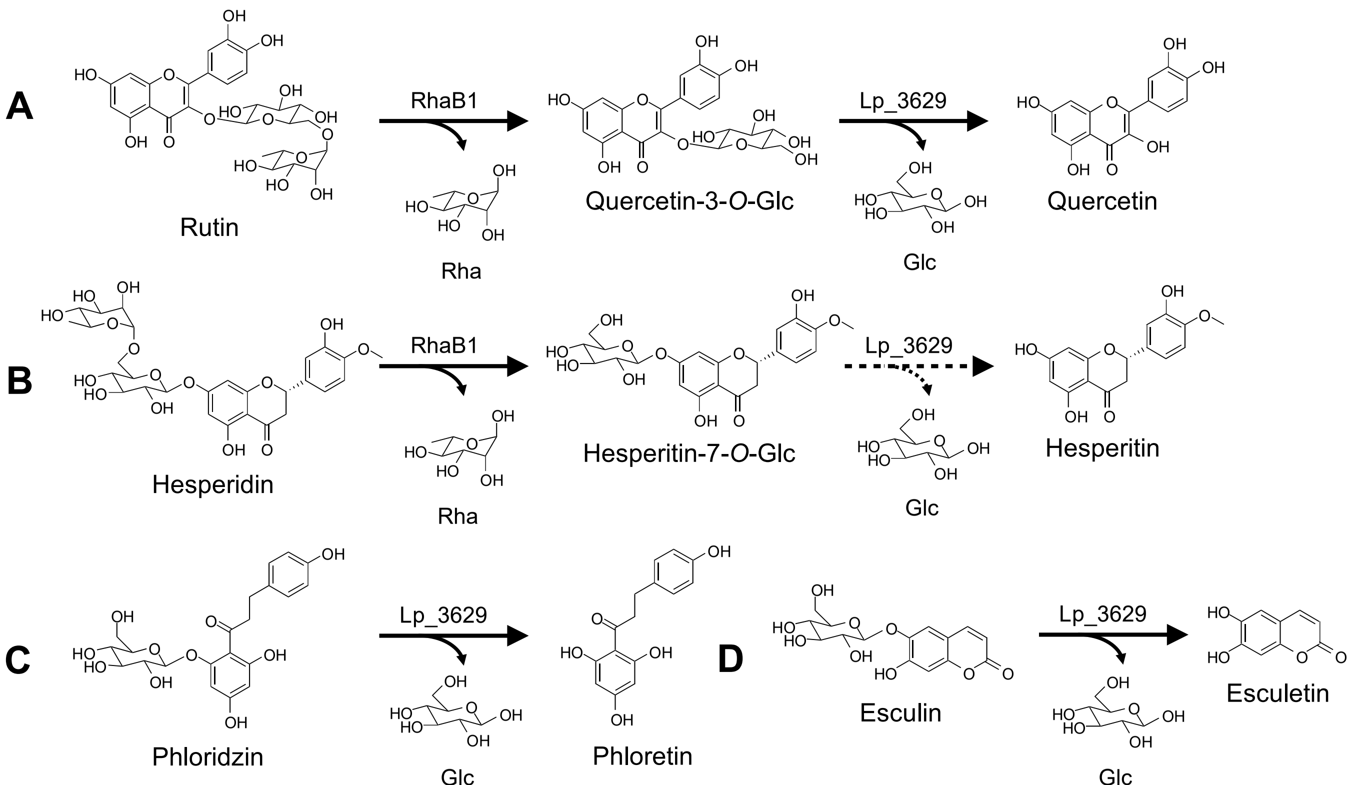


Fig. 10. Hydrolysis of rutin (A), hesperidin (B), phloridzin (C), and esculin (D) *via* α-rhamnosidases RhaB1B2 [102] and aryl glycosidase Lp_3629 [101]. Dashed arrow is a speculative pathway of hesperetin-7-*O*-glucoside hydrolysis. For details of the enzymes and genes, see **Supplementary Table S1**.

In theory, the activity of RhaB1B2 could be complemented by Lp_3629, as the removal of α-rhamnose transforms the compound from rutinoside to β-D-glucoside, and thus becomes a potential substrate for glycosidase Lp_3629, leading to the formation of a phenolic aglycone.

However, in the fermentation of fruit materials, flavonol glycosides and other potential substrates of microbial glycosidases often remain largely unaffected [103]. For example, β-glucosidase-catalyzed bioconversion of anthocyanins was reduced by 65 % in the presence of residual sugar [104]. Recently, it was observed that the β-glucosidase activity of *L. plantarum* UNQLp 11 was reduced by low pH (3.2 vs. 3.8), but induced by high ethanol content [105]. In addition, *L. plantarum* β-glucosidases were inhibited by low pH, ethanol, and sugars [33]. These factors together (i.e., low ethanol content, high sugar content, and low pH) could explain why phenolic glycosides are poorly metabolized by *L. plantarum* in non-alcoholic fruit material.

2.5.3.2 Benzyl alcohol dehydrogenase

While uncommon in lactic acid bacteria, the gene for benzyl alcohol dehydrogenase enzyme (*lp_3054*) was identified from *L. plantarum* WCFS1 by Kleerebezem et al. [20]. Later, the *lp_3054* protein was genetically and biochemically characterized by Landete et al. [106]. As aromatic alcohols are important odor compounds, Lp_3054 is a potential enzyme for flavor modification to reversibly oxidize benzyl alcohols to aldehydes with almond, green and grain odors. The enzyme can also reduce *cis*- and *trans*-geraniol (floral aromas) to citral A and citral B with a citrus aroma, respectively (**Fig. 11**).

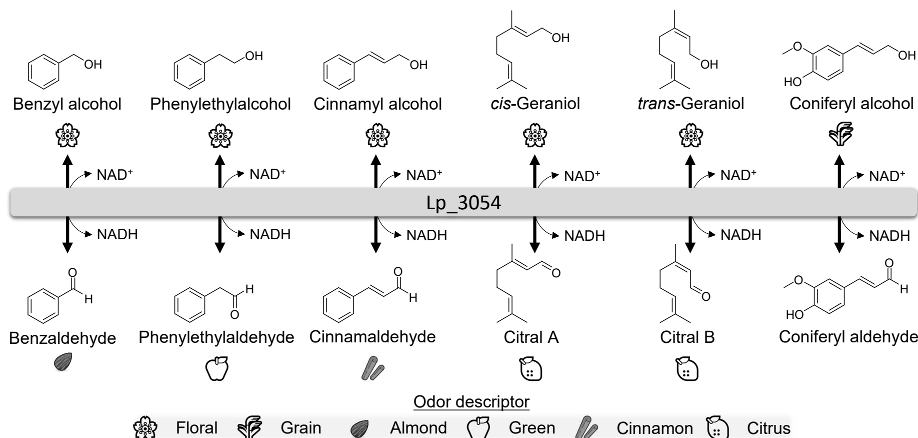


Fig. 11. Substrates and products of the benzyl alcohol dehydrogenase (Lp_3054) [106] and the typical odor descriptor of each compound [52].

2.6 Protein and amino acid metabolism in *L. plantarum*

2.6.1 Metabolism of poly- and oligopeptides

Protein catabolism is important for growth and function of micro-organisms, providing cells nitrogen and energy source as well as the essential amino acids the organisms are unable to produce themselves. Amino acids, whether derived from proteins and peptides or *de novo* synthesized by *L. plantarum*, however, are not only relevant as building blocks for enzymes and proteins, but also metabolized through various pathways into biogenic amines (**Section 2.6.3**), various odor compounds (**Section 2.6.2**) or antifungal compounds (**Section 2.6.5**). In addition, peptides, and amino acids, derived from proteolytic activity of lactic acid bacteria, have various flavor properties of their own. Therefore, understanding proteolytic systems and nitrogen metabolism of the fermentation organism is relevant not only for optimizing the biomass production or fermentation efficiency, but also for understanding or even predicting changes in the chemical composition and flavor properties of the raw material.

The first step in protein metabolism is to break down the polypeptide chain to smaller oligo-, tri and dipeptides with extracellular proteases. However, within the genus *Lactobacillus*, the gene for protease (such as PrtP or PrtM) is absent in most species, including *L. plantarum* [20,37,107]. While generally lacking the gene for catabolizing large polypeptides, *Lactobacillus plantarum* has an effective peptide transport systems [107,108]. Genomic analysis revealed that oligopeptide ABC transporters (*OppABCDF*) common in *Lactobacillus* were detected in *L. plantarum* 80, a strain isolated from spontaneous cocoa bean fermentation [37]. On the other hand, the *OppABCDF* system was missing from the strain *L. plantarum* strain WSCF1. However, copies of multiple di/tripeptide ABC transport systems (*DppABCDF*) were detected in this strain [107]. In addition to the before mentioned peptide transporters, a di/tripeptide ion-linked transporter (*DtpT*) was present in 66 of 71 tested *L. plantarum* strains [108].

Once inside the cell, peptides are hydrolyzed with an array of different peptidases. Several classes of peptidases, including aminopeptidases (*pepC*, *pepM*), endopeptidases (*pepO*), tripeptidase (*pepT*), and several proline peptidases (*pepI*, *pepX*) were found commonly present in *L. plantarum* [37,108]. From all tested peptidase genes, strain *L. plantarum* WSCF1 was only missing genes for PepA and Pcp, which hydrolyze Glu-Asp and dipeptides containing pyroglutamic acid, respectively [107].

While lactic acid bacteria and *L. plantarum* thrive in nutrient rich materials, *L. plantarum* WCFS1 has pathways for the biosynthesis of most of the amino acids. However, pathways for the branched chain amino acids (BcAA) valine, leucine and isoleucine were absent (**Table 3**) [20]. While *L. plantarum* contains pathway to produce the precursors required for BcAA biosynthesis, pyruvate and

acetolactate, the fact that it is missing the genes (*ilvC* and *ilvD*) required for conversion of acetolactate to the necessary α-ketoacids seems to be the cause of the auxotrophy [109]. In accordance with this, several genes for BcAA transporters were detected in genome of *L. plantarum* WCFS1 [20].

Glutamate auxotrophy is a common, even universal, trait among lactic acid bacteria. While *L. plantarum* possess a partial TCA cycle (**Fig. 3**), it is unable to produce the necessary precursor α-ketoglutarate [110]. *L. plantarum* WCFS1 contains four complete glutamine transport systems, which suggests that glutamine transport is important for regulating nitrogen metabolism [20].

Table 3. Growth rate of *L. plantarum* in a minimal medium when amino acids are omitted, in addition to the potential precursors in biosynthesis.

Amino acid	***Growth when omitted***		***Putative precursors in biosynthesis***[34,37,110,111]
	Morishita et al. 1981[111]	***Teusink et al. 2005***[110]	
Alanine	+*	95**	Pyruvate (glycolysis), asparate
Asparagine	+		Asparate
Glycine	+	68	
Asparatic acid	+	110	Oxaloacetic acid (TCA)
Proline	+	104	Arginine, ornithine
Serine	+	95	3-Phosphoserine, 3-phosphohydroxypyruvate
Phenylalanine	-	44	Chorismate (shikimate pathway), phenylpurvate
Lysine	+	105	
Tyrosine	+/-	70	Chorismate (shikimate pathway), hydroxyphenylpuryvate
Threonine	+	98	Asparate, homoserine
Isoleucine	-	23	
Tryptophan	-	20	Chorismate (shikimate pathway), indole
Arginine		2	
Glutamatic acid	-	1	α-Ketoglutarate
Glutamine	+		
Leucine	-	3	
Valine	-	4	
Histidine	+	93	Ribose-5-phosphate, histinidol
Cysteine	+	92	Sulfite, sulfide, serine
Methionine	-	41	Aspartate, cystathionine, homocysteine

* Plus-symbol means improved growth and minus-symbol means reduced growth.

** Growth rate in percentage compared to optimal conditions (100% at optimal).

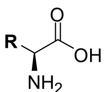
2.6.2 Metabolism of amino acids to flavor compounds

Amino acid metabolism provides an energy and nitrogen source for the cell. These metabolic pathways produce aroma-active compounds that are relevant to the flavor of fermented foods, especially of protein rich foods such as cheeses. Many amino acids, dipeptides, and tripeptides have inherent taste properties ranging from sweet to bitter and to umami and kokumi [112]. However, due to the lack of enzymatic activity to breakdown polypeptides, in fermentations using *L. plantarum*, catabolism of oligopeptides to amino acids and to further downstream compounds are the most relevant pathways in terms of modifying the flavor of the raw material.

One pathway for amino acid metabolism is transamination, where the amino group is transferred from an amino acid to α-ketoacid, yielding a new amino acid. In *L. plantarum* enzymes for transamination of BcAAs (BcAT, AsAT) and aromatic amino acids (ArAT) have been identified, where the amino acid yielded is glutamate from α-ketoglutarate. Ketoacids from BcAAs are precursors of various flavor-active alcohols, aldehydes, and carboxylic acids (**Fig. 12, Table 4**). In addition, non-enzymatic, Mn^{2+}-dependent formation of benzaldehyde (almond-like aroma) from phenylpyruvate, α-ketoacid of phenylalanine, was observed in *L. plantarum* LcL1. As large intracellular Mn^{2+} pool is required for the reaction, this pathway is specific to *L. plantarum* [113].

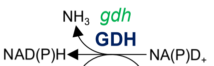
As pyruvate and oxaloacetate also possess the α-ketoacid structure, it has been proposed that α-ketoacids from BcAA are potential substrates for lactate, malate, and pyruvate dehydrogenases. In addition, in *Lactococcus lactis*, a D-2-hydroxyacid dehydrogenase with specific activity on BcAA α-ketoacids (PanE) has been identified [114]. While substrate specificity is yet to be determined, it has been speculated that *L. plantarum* could transform BcAA α-ketoacids into methyl propanoic and methyl butanoic acids through a pyruvate oxidation pathway; the typical activity of this pathway is to convert pyruvate into acetyl-CoA. An alternative pathway is through keto-acid decarboxylase (KDC) yielding aldehyde which is in turn oxidized to carboxylic acid by aldehyde dehydrogenase (**Fig. 12**). While [115] reported no copies of the KDC gene in *L. plantarum* WCFS1, activation of KDC pathway on BcAA ketoacid metabolism was suggested by [50] in fermented plant material. Alternatively, aldehyde produced *via* KDC enzyme can be reduced to the corresponding alcohol with NADH by alcohol dehydrogenase. Moreover, if the fermenting organism produces esterase with alcohol or acyl transferase activity (such as EstA), the carboxylic acids and alcohols formed through BcAA metabolism will provide potential substrates to ester biosynthesis (ester hydrolysis and formation is discussed in **Section 2.6**). Aldehydes from BcAA typically have an aldehydic aroma with chocolate notes, while alcohols in turn have a fermented or whiskey descriptor (**Table 4**).

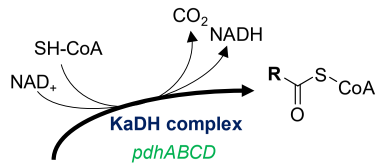
A



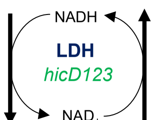
R	Compound
1	L-Valine
2	L-Leucine
3	L-isoleucine

R	Group
1	
2	
3	

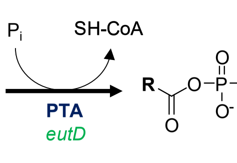




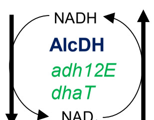
R	Compound
1	α-Ketoisovalerate
2	α-Ketoisocaproate
3	α-Keto-β-methylvalerate



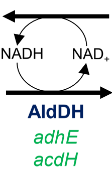
R	Compound
1	2-OH-isovalerate
2	2-OH-isocaproate
3	2-OH-3-methylvalerate

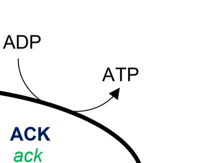


R	Compound
1	2-Methyl propanal
2	3-Methyl butanal
3	2-Methyl butanal

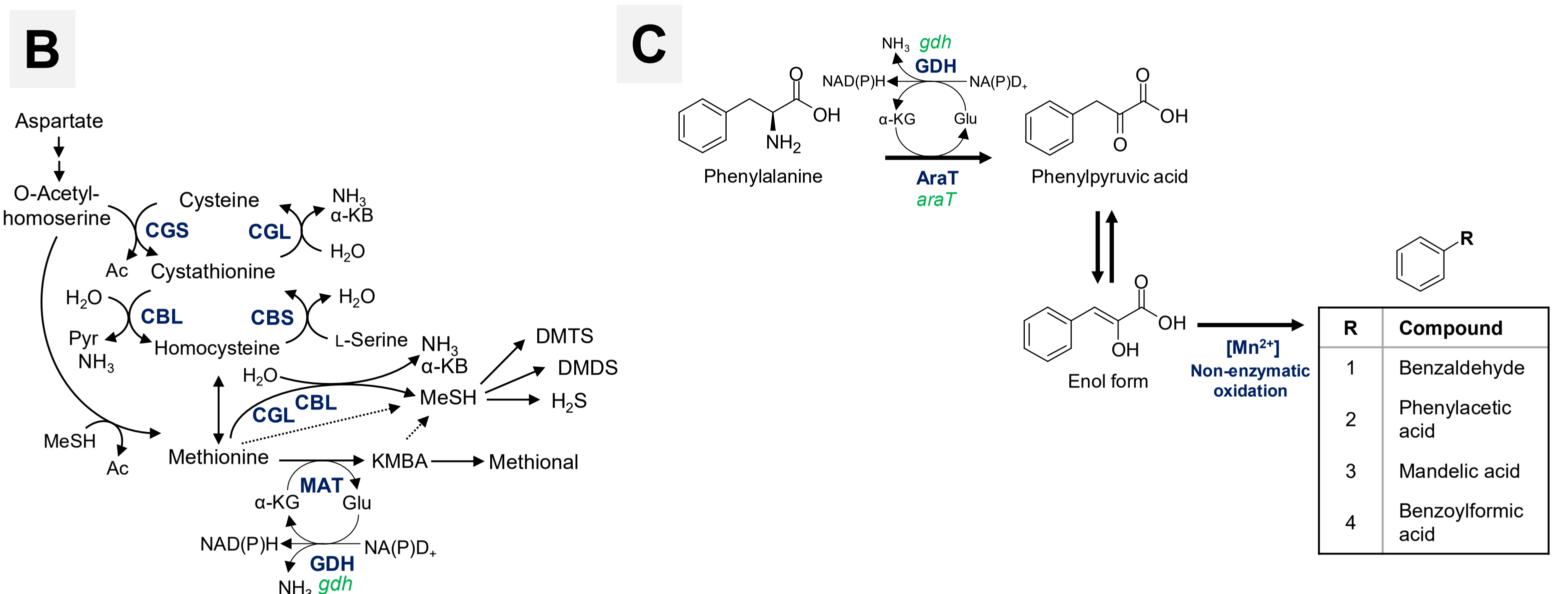


R	Compound
1	2-Methyl-1-propanol
2	3-Methyl-1-butanol
3	2-Methyl-1-butanol





R	Compound
1	2-Methyl propanoic acid
2	3-Methyl butanoic acid
3	2-Methyl butanoic acid



R	Compound
1	Benzaldehyde
2	Phenylacetic acid
3	Mandelic acid
4	Benzoylformic acid

Fig. 12. Metabolic pathways identified from *L. plantarum* related to the formation of various flavor compounds from A) branched chain amino acids, B) sulfur-containing amino acids, and C) phenylalanine. Adapted from [41,42,113,115–120]. Abbreviations: KMBA, 4-methylthio-2-ketobutanoate. For details of the enzymes and genes, see **Supplementary Table S1**.

Table 4. Typical odor descriptors of odor compounds derived from the free amino acid catabolism of *L. plantarum* [52].

Precursor	***Product***	***Odor descriptor***
Cysteine	Dihydrogensulfide	Rotten egg
Isoleucine	2-methyl butanal	Chocolate, cocoa, coffee
Isoleucine	2-methyl butanoic acid	Cheesy, dairy, fatty, fruity
Isoleucine	2-methyl-1-butanol	Roasted, onion, fruity, whiskey
Leucine	3-methyl butanal	Aldehydic, chocolate, peach
Leucine	3-methyl butanoic acid	Acidic, fruity, dirty, cheesy
Leucine	3-methyl-1-butanol	Fermented, whiskey, fruity, banana
Methionine	Methional	Vegetable, potato, earthy
Methionine, cysteine	Dimethyldisulfide	Sulfurous, cabbage, onion
Methionine, cysteine	Dimethyltrisulfide	Alliaceous, sulfurous
Methionine, cysteine	Methanethiol	Sulfurous, cabbage, garlic
Phenylalanine	Benzaldehyde	Fruity, bitter, almond, cherry
Phenylalanine	Phenylacetic acid	Honey, sweet, floral
Valine	2-methyl propanal	Aldehydic, fresh, herbal
Valine	2-methyl propanoic acid	Acidic, sour, cheese, dairy
Valine	2-methyl-1-propanol	Ethereal, whiskey

BcAA-derived carboxylic acids have acidic and cheese aromas and are relevant flavor compounds in cheeses [112].

In addition to transamination of BCAA, other metabolic pathways of amino acid metabolism can be considered relevant for flavor formation in fermentation with *L. plantarum*. Serine (through dehydration and deamination), and asparate and alanine (through transamination) are potential precursors for pyruvate. In addition, threonine, a potential precursor for pyruvate and acetaldehyde, was proposed to be metabolized by *L. plantarum* through the same metabolic pathways as serine [37]. However, while serine was metabolized by *L. plantarum* B3089 to ammonia, acetate and formate, no threonine catabolism was detected [121].

Metabolism of sulfur-containing amino acids (methionine, cysteine) produce compounds with cabbage, onion, and sulfurous notes. To form these compounds, methionine is converted first to MeSH which in turn works as precursor for various volatile sulfides. However, no copies for the gene of the enzyme particularly catalyzing this reaction, methionine γ-lyase, exist in *L. plantarum*. Instead, cystathione β/γ-lyase can also catalyze this reaction, but less effectively [115,119]. It was observed that the main pathway for methionine modification in *L. plantarum* was transamination to 4-methylthio-2-ketobutanoate (KMBA) and further reduction to 4-methylthio-2-hydroxybutanoate (HMBA) [119]. Even lacking methionine γ-lyase activity, *L. plantarum* was reported to produce sulfur

volatiles [117]. Therefore, it is possible that the sulfur compounds are formed due to the non-enzymatic reactions of HMBA and KMBA in the presence PLP.

Volatile sulfides were also produced also in the presence of cysteine, suggesting conversion of cysteine to methionine *via* cysthathionine [117]. Interestingly, while *O*-succinyl-homoserine is typically presented as co-substrate along with cysteine for cystathionine γ-synthase [120], this compound requires succinyl-CoA in its biosynthesis; the current assumption is that *L. plantarum* is unable to produce this compound [56]. Instead, in *L. plantarum*, an alternative substrate for cysteine to methionine conversion could be *O*-acetyl-homoserine.

Formation of *N*-heterocycles with a mouse-like odor by *L. plantarum* from L-lysine and L-arginine is discussed in **Section 2.8.4**.

2.6.3 Metabolism of amino acids to biogenic amines and ethyl carbamate

As discussed in the previous section, amino acid metabolism is important for flavor formation in certain lactic acid fermented foods. However, food-grade lactic acid acid bacteria are known to produce unwanted, even toxic compounds, i.e., ethyl carbamate and biogenic amines. The former is especially related to arginine metabolism and is formed in a reaction between ethanol and *N*-carbamyl compounds such as carbamyl phosphate and citrulline [122]. Biogenic amines on the other hand are a product of decarboxylation of amino acids arginine, tyrosine, histidine, lysine, and ornithine (**Fig. 13**). While ethyl carbamate is a known carcinogen due to covalently binding to DNA, biogenic amines have an array of undesirable biological activities [123]

While *L. plantarum* strains often possess genes for amino acid decarboxylation, an accumulation of toxic levels of biogenic amines is usually due to contamination of for example *Enterobacteriaceae* or food-borne pathogens [124]. Biogenic amine formation by spoilage micro-organism can be either induced or inhibited by the starter culture *L. plantarum* depending on the strain [125]. In general, availability of the substrate amino acid, spontaneous fermentation, and acid stress or glucose depletion (i.e. conditions that promote amino acid utilization) induce biogenic amine formation, while salting seems to inhibit formation of biogenic amines by either preventing growth of biogenic amine-forming bacteria or directly inhibiting amino acid decarboxylase enzymes [126,127]. However, more frequently toxic levels of biogenic amines are related to poor manufacturing practices rather than suboptimal fermentation conditions [125].

Lack of biogenic amine formation is an important characteristic for wine malolactic starter, and thus this characteristic is often determined when new wine MLF starters are screened [53].

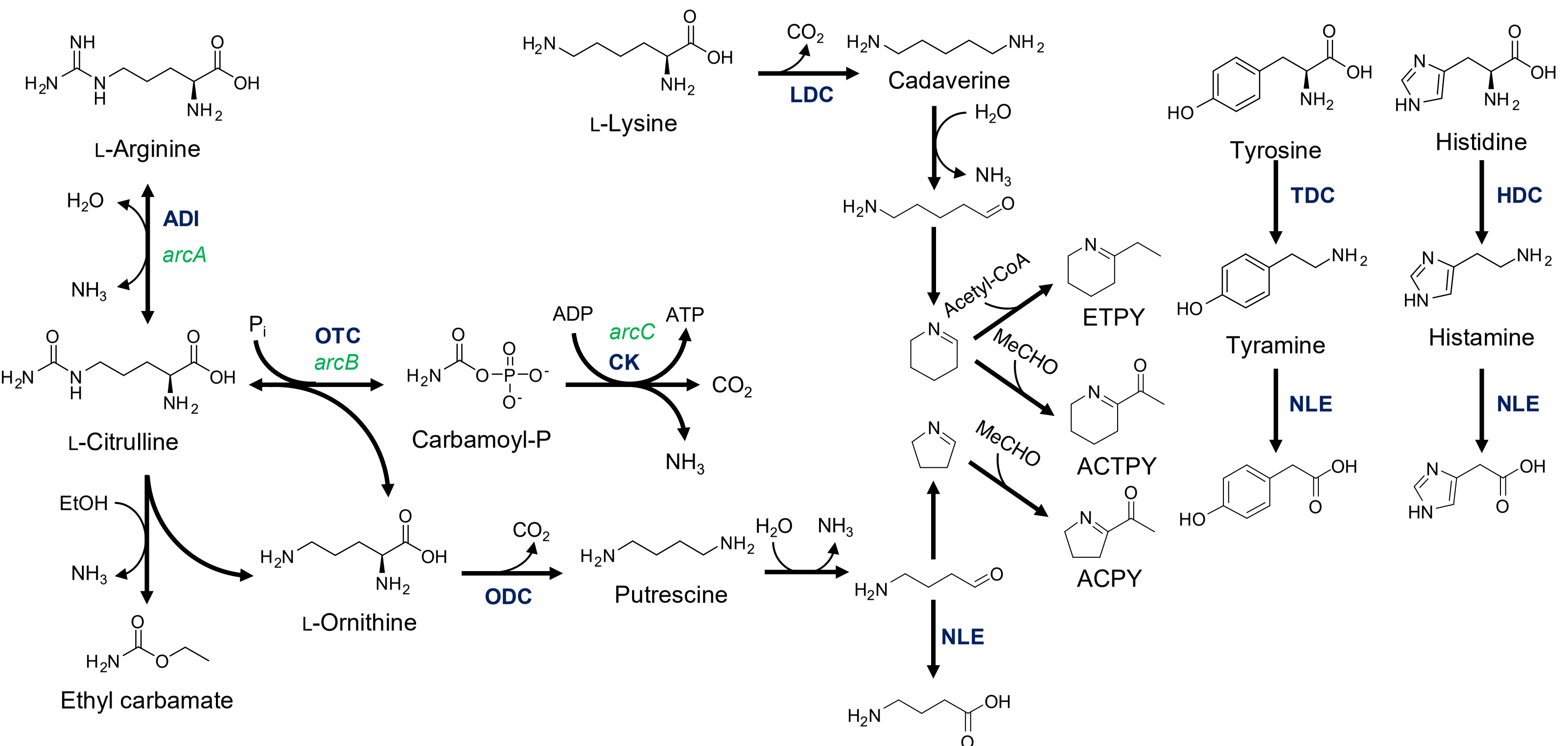


Fig. 13. Ethyl carbamate, biogenic amine, and *N*-heterocycle formation in *L. plantatrum* [128–133]. For details of the enzymes and genes, see **Supplementary Table S1**.

While for wine MLF formation biogenic amines and ethyl carbamate precursors are undesirable, in sourdough fermentation, accumulation of ornithine is desirable as it is a precursor of 2-acetyl-1-pyrroline, which is an important flavor compound in bread [134].

Depending on the strain, *L. plantarum* can be a source of biogenic amines, especially tyramine and histamine [135]. However, some strains *L. plantarum* have the ability to degrade biogenic amines. When 26 *L. plantarum* wine isolates were screened, two isolates, NDT09 and NDT16, showed substantial degradation of tyramine and putrescine, respectively [136]. An enzyme with the ability to degrade biogenic amines via oxidation was isolated from strain J16 CECT 8944 and identified as a laccase (multicopper oxidase) by Callejón et al. (**Fig. 13**) [137]. Potential biogenic amine degrading candidates, namely 10 amine oxidase enzymes were identified from *L. plantarum* CAU 3823. These were identified as different amine and monoamine oxidases [138]. In addition, a putative histamine degrading glyceraldehyde-3-phosphate dehydrogenase was isolated from *L. plantarum* PP02 [139].

In relation to fermentation of acidic materials, such as berries or sour fruits, the increased acidity tolerance in *L. plantarum* has been associated with enhanced amino acid utilization as an alternative energy source and to maintain pH homeostasis [140]. However, utilization of such strains for fermentation has potentially increased the risk of biogenic amine accumulation. A potential solution could be to co-inoculate the raw material with biogenic amine degrading strain to avoid accumulation of these toxic compounds during fermentation.

2.6.4 Metabolism of amino acids to *N*-heterocycles

N-heterocycle contamination is not a common issue in the MLF of wines, but when it occurs, it renders the wine unpalatable by producing a mouse-like off-odor in the wine. The most common ones detected in wines are 2-ethyltetrahydropyridine (ETPY), 2-acetyltetrahydropyridine (ACTPY) and 2-acetyl-1-pyrroline (ACPY) (**Fig. 13**). Comparison of various lactic acid bacteria revealed that heterofermentive *Lactobacillus* produce higher amounts of these compounds compared to *Oenococcus* spp. and *Pediococcus* spp. Among *Lactobacillus*, facultative homofermentive *L. plantarum* L11a produced detectable yet low amounts of all previously mentioned *N*-heterocycles, linking formation of these compounds to sugar catabolism [129]. A later study presented a putative synthesis pathway to N-heterocycles (**Fig. 13**), where L-lysine was suggested as precursor for ETPY and ACTPY and L-ornithine for ACPY. Acylation group was proposed to be ethanol and acetaldehyde, produced from glucose or fructose *via* phosphoketolase pathway, explaining why *N*-heterocycles were produced in higher amounts in heterofermentive *Lactobacillus* compared to homofermentive species within the same genus [129]. Furthermore,

presence of ferrous ions was recognized as important for the reaction to initiate. Other risk factors were residual sugar or stuck fermentation, high pH, minimal sulfite content and moderate temperature (20–30 °C) [130].

While food fermentations with *L. plantarum* seem to have a low risk for *N*-heterocycle contamination, there are clear risk factors that should be considered. Firstly, the presence genes of *arcA* and *arcB* in the *L. plantarum* strain that allow conversion of arginine to L-ornithine [133]; Secondly, an environment that increases the metabolic flux to heterolactic pathways of *L. plantarum* (for example, high content of sugar alcohols [39]); Thirdly, the risks mentioned above, as identified by [130].

2.6.5 Metabolism of amino acids to antifungal compounds

Multiple antifungal compounds produced by *L. plantarum* have been identified, such as phenyllactic acid (PLA), hydroxyphenyllactic acid (OH-PLA), indole lactic acid (ILA), cyclic dipeptides and 3-hydroxy fatty acids (**Table 5**) [141,142]. Except for the 3-hydroxy fatty acids, the previously mentioned compounds are produced through pathways related to protein metabolism. For PLA, OH-PLA and ILA, the precursors are the aromatic amino acids phenylalanine, tyrosine, or tryptophan, respectively. The amino acids are first converted to keto-acids through transamination, and further reduced to corresponding compounds. Alternatively, the necessary precursors can also be produced through a shikimate pathway [143]. Cyclic depeptides are formed through the condensation of two amino acids. No putative biosynthesis route for methylhydantoin or mevalonolactone have been suggested. The structure of methylhydantoin, however, suggests similar mechanism to cyclic dipeptide formation where the substrates for condensation would be alanine and carbamoyl-phosphate or alanine and citrulline [144].

Antifungal compounds produced by *L. plantarum* have shown to inhibit growth of *Aspergillus* and *Penicillium* as well as inhibit aflatoxin production of *Aspergillus* [145]. In addition, a few studies have shown effectiveness with *Fusarium* as well (**Table 5**). When antifungal activity was compared between PLA, OH-PLA and ILA, only PLA inhibited growth of *A. flavus* (IC90 11.9 mg/mL) at tested concentration [145]. Additionally, the antimicrobial activity of PLA was augmented by both lactic and acetic acid [146]. Cell-free supernatants of *L. plantarum* lost antifungal [23] and antimicrobial [22] activity after pH neutralization, suggesting that the previously mentioned organic acids are relevant for the antifungal activity of *L. plantarum* [23].

Regarding the antimicrobial potential of *L. plantarum* derived antifungals, pure compounds could be used as novel antimicrobials; alternatively, antifungal producing strain can be used as natural preservatives. In fact, sourdough produced with *L. plantarum* FST 1.7 was able to retard growth of *Fusarium* [141].

2.6.6 Bacteriocin biosynthesis in *L. plantarum*

Lactic acid bacteria have numerous mechanisms to outcompete other micro-organisms. One such mechanism is the biosynthesis of antimicrobial peptides, known as bacteriocins. Depending on the chemical composition, size and mechanism of action, bacteriocins are divided into classes I, II and III, with class II further divided into four different subclasses IIa-IId (**Table 6**) [147].

Table 5. Antifungal compounds produced by various *L. plantarum* strains and the antifungal activity of the compounds.

Strain	***Origin***	***Antifungal compound***	***Effective against***	***Ref.***
L. plantarum UM55		Phenyllactic acid, hydroxylphenyllactic acid, indole lactic acid	*Aspergillus*	145
L. plantarum FST 1.7	Malted barley	Phenyllactic acid, cyclo(Leu-Pro), cyclo(Phe-Pro)	*Aspergillus niger*, *Fusarium* sp.	141
L. plantarum AF1	Kimchi	cyclo(Leu-Leu)	*Aspergillus, Penicillium, Epicoccum*, *Cladosporium*	148
L. plantarum MiLAB 14	Lilac flowers	Hydroxy fatty acids	*Aspergillus*, *Penicillium*	142
L. plantarum VTTE 78076	Beer	Benzoic acid, methylhydantoin, mevalonolactone, cyclo(Gly-Leu)	*Fusarium avenaceum*	144

Bacteriocins detected from *L. plantarum* are known as plantaricins. Plantaricins are typically most effective against Gram-positive bacteria, and moderately effective against selected Gram-negative bacteria (such as *E. coli*). Their antifungal properties are poorly reported. While the list is not exhaustive, plantaricins often seem to be small peptides, ranging between 1–3 kDa in size (**Table 6**).

Analysis of sixteen *L. plantarum* strains isolated from table olives and brine showed that among the genes belonging to the *pln* locus, *plnG* and *plnC* were the most prevalent genes, followed by *plnD* and *plnB*. The genes *plnE*/*F* and *plnI* were detected in half of the studied strains [149]. The plantaricin production regulators identified so far are the operons *plnABCD* and *plNC8-plNC8HK* which are controlled by the autoinducer peptides, PlnA1 or PLNC8IF, respectively [150,151]. PlnA1 activates histidine kinase PlnB1, leading to phosphorylation of PlnC or PlnD, which in turn either activate or repress the expression of the *pln* locus, respectively [152].

Table 6. Classifications of types of A) bacteriocins [147] as well as B) source, properties, and antimicrobial activity with Gram(+) bacteria, Gram(-) bacteria and fungi of various plantaricins.

A

Class	***Name***	***Characteristic***	***Size***	***Other properties***
I	Lantibiotics Type A and B	Lanthionine-containing	<5 kDa, 19–38 AA	Type A: pore forming. Type B: Enzyme inhibitors
IIa	Listeria-active bacteriocins	Active against Listeria spp., conserved amino acid sequence, YGNGV	< 10 kDa, no unusual AA	Heat-stable, membrane-active
IIb	Two-peptide complexes	Two synergistic peptides	< 10 kDa, no unusual AA	Pore-forming, pH gradient dissipation
IIc	The *sec*-dependent bacteriocins	a *sec*-type N-terminal leader sequence, leading to secretion and processing via the *sec* pathway	< 10 kDa, no unusual AA	
IId	Unclassified small heat-stable non-lanthionine bacteriocins	Bacteriocins that do not meet category of any other in Class II.	< 10 kDa, no unusual AA	
III	Large heat-labile bacteriocins		> 30 kDa	

B

Name	***Source***	***Class***	***Size (kDa)***	***Amino acid sequence***	***Gram(+)***	***Gram(-)***	***Fungi***	***Ref.***
Bacteriocin SLG10	Kombucha	IId	1.42	NIVWQLIGLPAQAl	All tested	*E. coli* inhibited	No activity	153
Plantaricin K25	Kimchi		1.77		All tested	All tested	Not tested	154
Plantaricin GZ1-27	Chinese Kipper	II	0.98	VSGPAGPPGTH	*B. cereus*	Not tested	Not tested	155
Plantaricin JLA-9	Fermented cabbage		1.04	FWQKMSFA	All tested	All tested	Not tested	156

Abbreviation: AA, amino acid; *sec*, secretory.

Recently, acetate was discovered to be an additional activator of histidine kinase PlnB1 [157]. PLNC8IF, on the other hand, activates histidine kinase PLNC8HK, leading to phosphorylation of PlnD.

However, unlike the *plnABCD* operon, PlnD works as an activator rather than repressor for the *plNC8-plNC8HK* system [151]. The third system related to the histidine kinase AgrC, activated by autoinducer-2 (AI-2), was not directly associated with bacterocin biosynthesis. However, it was speculated that this pathway activates metabolic systems that allow bacteriocin biosynthesis i.e., amino acid, carbohydrate, and fatty acid metabolism [158].

Regardless of the plantaricin biosynthesis system present in the *L. plantarum* strain, plantaricin production is regulated by quorum sensing. It has been observed that the cell density of both *L. plantarum* and competing micro-organism(s) need to be high enough for the threshold for the quorum sensing system to be activated which in turn activates plantaricin production [159].

Plantaricins are potential novel food antimicrobials. However, the ability to maintain tertiary structure during food processing is important for peptide-based antimicrobials. Several plantaricins have showed high thermal, pH, and enzymatic stability [154,155]. Besides using the pure compound as a food additive, another possibility is to increase shelf-life by using the strain to ferment raw material that is known to produce an effective plantaricin. However, the strain would need to synthesize the plantaricin at adequate levels to have any practical significance for preservative purposes. As plantaricin biosynthesis is regulated by quorum sensing, bacteriocin production can be induced by co-culturing *L. plantarum* with another microbial species. For example, more than a 10-fold increase in bacteriocin production was observed in *L. plantarum* CECT4185 after co-culturing with *Lactococcus lactis* IL1403 [151]. The food matrix is also relevant as plantaricin production was induced in solid food material even at low inoculation levels, while inoculation of 9 log CFU/mL of *L. plantarum* C2 was required to produce detectable plantaricin activity in carrot juice [160]. Therefore, when using a monoculture of *L. plantarum* to ferment pasteurized vegetable or fruit juices only limited plantaricin production can be expected. In these circumstances, one option would be to add exogenous autoinducer peptide (e.g., PLNC8IF) if plantaricin activity is desired [159]. However, it should be taken into account that plantaricin production takes away resources from other cellular functions, and therefore may have a negative effect on fermentation especially if the medium or the raw material has poor nutrient quality [159].

2.7 Stress responses of *L. plantarum* relevant to plant fermentations

For *L. plantarum* to adapt to a variety of plant niches, genes for sensing the environment and adapting to biotic and abiotic stress are necessary (**Fig. 14**). In the MLF of wines, abilities to tolerate low pH, high ethanol content, phenolic acids, and sulfites are necessary. As vegetables and mushrooms are typically fermented in brine, protection from osmotic stress becomes relevant, while protective metabolic responses to shocks from cold temperature (during food storage), stomach acids, and bile acids are important characteristics for probiotic *L. plantarum* strains [9].

Transcriptional regulators CtsR and HrcA play a key role in the universal stress response of *L. plantarum*, and are relevant for stress adaptation to e.g. heat [161], cold [162], and ethanol [58]. CtsR and HrcA regulate expression of heat-shock proteins (*hsp1*, *grpE*, *dnaK*), intracellular proteases (*clpC*, *clpP*), and chaperonin (*dnaJ*, *groEL*, *groES*) [163]. The role of a intracellular protease is to degrade nonfunctional proteins while chaperonin aid the folding of proteins during cellular stress [20].

In wine MLF, it was observed that stress-related gene (*hsp1*, *hsp2*, *ctsR*) expression in *L. plantarum* correlated with the ability to tolerate low pH [164]. In addition, overproduction of Hsp 18.55 and Hsp 19.3 in *L. plantarum* WCFS1 led to an enhanced survival in the presence of butanol (1%, v/v) or ethanol (12%, v/v) [162]. Heat-shock proteins also seem to play a role in probiosis of *L. plantarum* as *hsp* knockout mutants showed reduced resistance to oro-gastro-intestinal stress, adhesion to enterocytes, and immuno-modulation of macrophages [165].

The antimicrobial properties of ethanol and phenolic acids are due interaction with the lipid bilayer, which increases membrane fluidity and proton permeability, disrupting cell functions and ultimately leading to a loss of intracellular compounds and cell death [166]. Phenolic compounds, especially certain phenolic acids, are effectively metabolized by *L. plantarum* as a detoxification mechanism. Under *p*-coumaric stress especially, the *pdc* gene (*lp_3665*) was upregulated 112-fold along with increased methionine production [93]. Phenolic compound metabolism is discussed in **Section 2.7**.

Despite the high toxicity of ethanol on micro-organisms due to detrimental membrane interaction, certain wine lactic acid bacteria including *L. plantarum* and *Oenococcus oeni* have an ethanol tolerance of up to 14% (v/v) [167]. Exposure to ethanol induces a variety of changes in the metabolism of ethanol-resistant microbes, and 172 genes were found to be differently expressed in *L. plantarum* NF92 after ethanol treatment. Transcription factor AcrR was found to be important for ethanol tolerance, as overexpression of AcrR promoted growth of *L. plantarum* NF92 in 9% EtOH.

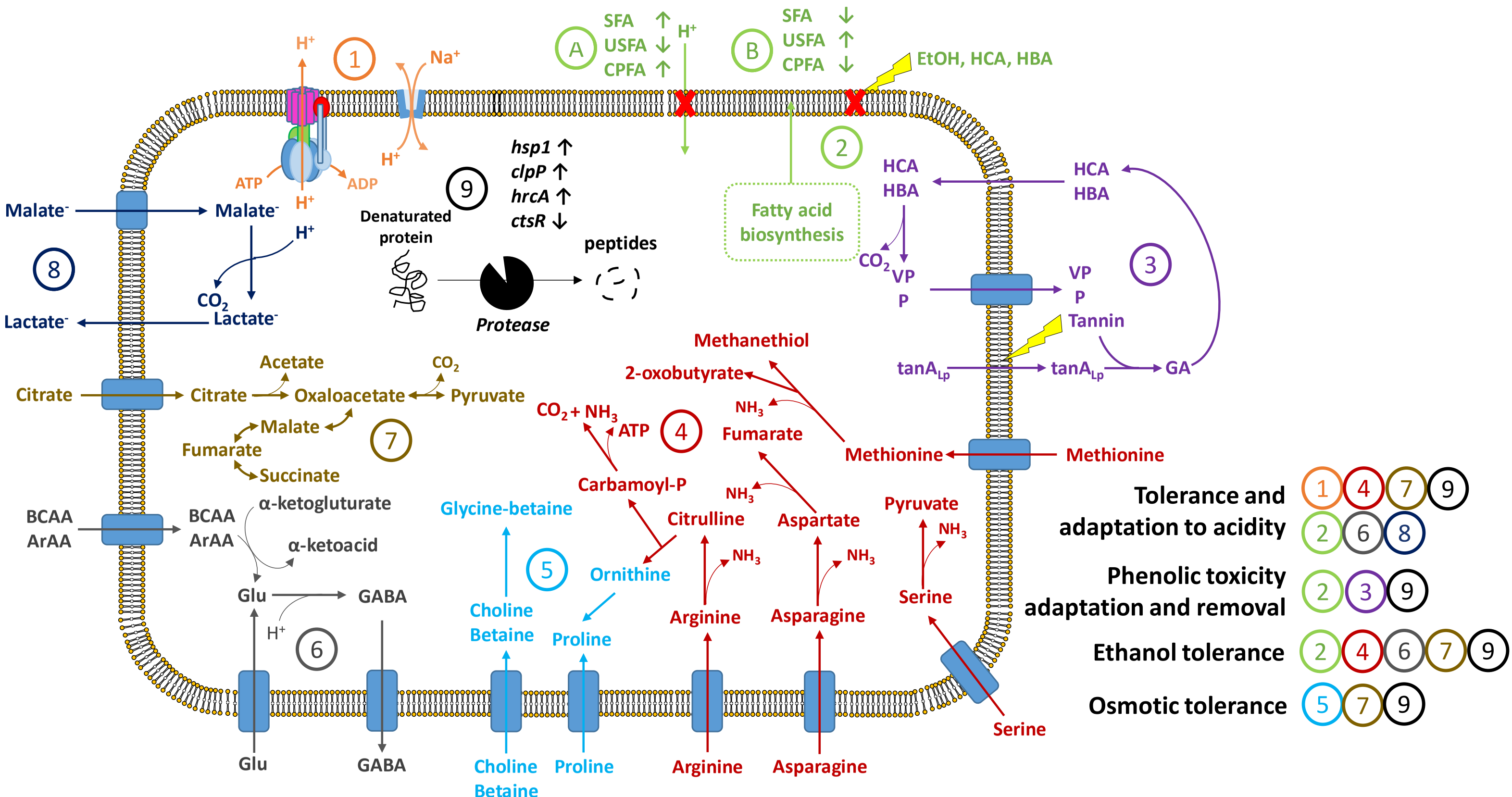


Fig. 14. Adaptation mechanisms of *L. plantarum* to various biotic and abiotic stress factors. 1: ATP-dependent F_0F_1-ATPase and sodium-proton antiporter to maintain pH homeostasis [20]. (Caption continued the following page)

2: Transformation of the microbial cell wall fatty acid composition to increase the rigidity to reduce proton influx (A) or to decrease the rigidity to counter the disruptive effects of ethanol or phenolic acids (B) (SFA, saturated fatty acid; UNFA, unsaturated fatty acid; CPFA; cyclo-propane fatty acid) [58]. 3: In some *L. plantarum* strains, extracellular tannase $tanA_{Lp}$ can hydrolyze tannins to reduce their toxicity, while hydroxybenzoic (HBA) and hydroxycinnamic (HCA) acids toxic to *L. plantarum* can be decarboxylated to less toxic phenol (P) or vinyl phenol (VP) derivatives, respectively [83]. 4: Metabolism of some amino acids yields NH_3 to neutralize intracellular protons [140]. 5: Accumulation of osmoprotectants (i.e., proline and glycine-betaine) under high osmotic stress [168]. 6: Transamination of branched-chain (BcAA) and aromatic amino acids (ArAA) to glutamate (Glu), and subsequent decarboxylation to γ-aminobyturic acid (GABA) [169]. 7: Citrate metabolism to pyruvate, or to succinate through the partial tricarboxylic acid cycle [58]. 8: Proton efflux due to the activity of the malolactic enzyme [63]. 9: General stress response by activation of HrcA which in turn upregulates genes for the heat-shock proteins (*hsp*) and the intracellular proteases (*clpP*) [20].

The key genes that were upregulated by AcrR were *fabZ1* (unsaturated fatty acid biosynthesis), *murD* (peptidoglycan biosynthesis) and *trmFO* (protein O-glycosylation). At the same time, the genes *cfa1*, *cfa2* and *tagE6* were downregulated by AcrR. Genes *cfa12* encode cyclopropane synthases, and the enzymes are associated with lactobacillic acid biosynthesis (**Fig. 15**) [43], which is produced from *cis*-vaccenic acid (18:1, n7, *cis*) [170].

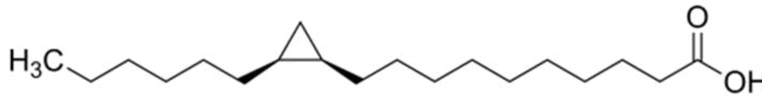


Fig. 15. Structure of the lactobacillic acid [170].

Both an increase in unsaturated fatty acid biosynthesis and a reduction in formation of lactobacillic acid increased membrane fluidity, which was concluded to be a mechanism to counter ethanol toxicity; a more fluid membrane is less susceptible to the disruptive effects of ethanol to membrane integrity [43]. Conversely, van Bokhorst-van de Veen et al. [58] reported that the ratio of unsaturated and saturated membrane fatty acids was reduced in *L. plantarum* WCFS1 when exposed to 8% EtOH.

Acid adaptation and tolerance are not only relevant to the fermentation of acidic plant or berry materials, but also for the storage stability and probiotic potential of the strain (i.e., ability to tolerate stomach acids), and thus acid tolerance is an important characteristic when screening functional strains for the food industry. In general, *L. plantarum* has a good acid tolerance and an ability to maintain moderate growth rates at pH 3.5 [140] while the optimal growth pH is around 6. The ability to tolerate low pH is related to the ability to maintain intracellular proton homeostasis. Efflux of excess H^+ can be mediated by F_0F_1-ATPase and sodium-proton antiporters [20]. While under ethanol stress membrane fluidity was increased [43], *L. plantarum* have been reported to decrease membrane fluidity as a response to acid stress; rigid membrane reduces proton flow into the cell. During storage in acidic fruit juice, the membrane rigidity of *L. plantarum* NCMIB 8826 was enhanced by increasing the biosynthesis of saturated and lactobacillic acid, along with a significant upregulation of cyclopropane synthase (*cfa*) [171].

In *L. plantarum*, amino acid accumulation and metabolism was increased during acid stress [140] while it was decreased under ethanol stress [58]. Increased transport of proline was detected as a response to osmotic stress [168]. As a results of metabolism of e.g. serine, arginine and methionine, intracellular pH is increased due to decarboxylation and release of ammonia [140]. Additionally, decarboxylation of glutamate yields γ-aminobyturic acid (GABA), and thus, GABA is often detected in fermented foods [169]. When acid tolerance of *L.*

plantarum strains ZDY 2013 (acid tolerant) and ATCC 8014 were compared, higher intracellular amino acid concentrations were observed in the former compared to the latter [140].

Metabolic pathways related to citrate metabolism were activated under stress from ethanol [58] and a low pH [51]. In addition, conversion of citrate-to-succinate was increased by high salinity [57]. The ability of citrate metabolism to generate membrane potential (e.g. citrate-sodium symport activity of CitP) and pH gradient were suggested as explanations of the pathway activation under ethanol stress [58]. In addition, some *L. plantarum* strains, such as NCU116, also express a membrane-bound oxaloacetate decarboxylase (*oad*), which allows conversion of citrate-derived oxaloacetate into pyruvate and CO_2 while using energy derived from the decarboxylation to transport Na^+ out of the cell [34].

One additional mechanism that enhances adaption to low pH is MLF; the pH of the raw material is increased due to the decarboxylation of L-malate [172]. In addition, efflux of lactate generates an electrochemical gradient (i.e., proton motive force) across the cell membrane [63]. Similar to amino acid metabolism, low pH seems to increase the metabolism of organic acids over carbohydrates in *L. plantarum* [103].

Lyophilized cells are the most practical way for the food and alcohol industry to store, transport, and use microorganisms in fermentation. However, freeze-drying damages bacterial cell walls and thus lyophilized cells are more sensitive to environmental stress compared to metabolically active cells. This in turn may lead to problems initiating healthy fermentation in challenging materials such as wines or berry juices with lyophilized cultures. However, it has been shown that acclimation of *L. plantarum* UNQLp155 cells at 6 % EtOH prior to freeze-drying improved adaptation to the freeze-drying process as well as leading to better growth in synthetic wine [173].

2.8 Fermentation of plant and fungi material with *L. plantarum*

2.8.1 Vegetables and mushrooms

Table olives, cabbage, cauliflower, and mushroom can be preserved *via* spontaneous lactic acid fermentation. Typically, the material is washed, followed by submerging in brine with an additional carbon source (such as sucrose). The fermentation of vegetable materials is started at ambient temperature but finalized at sub-ambient conditions (~16 °C) [174,175]. However, fermentation of materials such as table olives maintain ambient temperature throughout the fermentation [176]. Compared to the fermentation of fruit and berry materials (**Section 2.8.3**), fermentation times of vegetable materials tend to be

considerably longer, ranging from a month up to a year, as is the case with table olives [17,175,177].

As vegetables and mushrooms usually have a low initial acidity, the conditions at the start of fermentation benefit the mesophilic, halotolerant micro-organisms, but after acidification, the raw material becomes a hostile environment and only micro-organisms able to tolerate both high acidity and salinity can thrive. While each spontaneous fermentation has its own unique microflora, studies related to bacterial community development in vegetable fermentation have revealed several key genus and key species. The main contributor to initial acidification in spontaneous vegetable fermentation is *Leuconostoc* spp., especially *Leuconostoc mesenteroides*. When the pH falls below 3.5-4, *Lactobacillus* spp., especially *L. plantarum*, *L. brevis* or both, take over and finalize the fermentation [175,178–180].

When vegetable material was inoculated with *L. plantarum*, the species controlled the LAB fermentation throughout the process [178,180], leading to a more predictable fermentation process. This also reduces the risk of unwanted contaminations. However, when *L. lactis* was used as starter culture, the main species at the end of fermentation was still *L. plantarum* [178]. Additionally, using a starter culture instead of spontaneous fermentation generally leads to lower biogenic amine formation [178]. Strain-dependent properties can also provide further benefits: fermentation of shiitake mushrooms with *L. plantarum* GDM1.191 increased levels of the umami compounds [181] while fermentation of pea-protein isolate with *L. plantarum* was an effective method for off-aroma removal [182].

2.8.2 Wine malolactic fermentation

For the *L. plantarum* strain to be utilized as wine MLF starter, it should possess very specific traits. Wine being a hostile environment for micro-organisms, the fermentation organism is required to tolerate stress from a high ethanol content, a low pH, SO2 and lysozyme. Besides ability to survive in wine conditions, the micro-organism should be able to metabolize L-malic acid and citric acid. Regarding the metabolism of wine phenolics, tannase and phenolic acid decarboxylase activity are often screened. While the former allows removal of gallotannin, which reduces astringency and haziness in wine, the latter enzyme produces volatile phenols. Whether phenolic acid decarboxylase activity has positive or negative impact on the sensory value of wine, depends on the substrate (**Section 2.5.2**).

While amino acid decarboxylation enhances the pH tolerance of *L. plantarum* [140], accumulation of biogenic amines is considered a negative trait in an MLF starter. Therefore, especially histidine and tyramine decarboxylase activities are often screened. To further develop the wine *bouquet*, β-glucosidase and proline

aminopeptidase activity are desired to release bound volatiles from glycosides or amino acids, respectively [183]. Finally, esterases, especially when accompanied with alcoholtransferase activity, are sought after in wine malolactic starters to increase the number of fruity esters in the wine [75].

When 53 wine isolates from Patagonian red wine were analyzed, two strains, *L. plantarum* UNQLp 97 and UNQLp 155 showed promise as wine MLF starters [167]. The former strain exhibited good acid tolerance, while the latter maintained almost a full relative growth rate (95%) even at 14% (v/v) EtOH. Both strains consumed L-malic acid and degraded gallotannin effectively, and in addition, *L. plantarum* UNQLp 97 showed high β-glucosidase activity as well. In another instance, *L. plantarum* isolates from Patagonian pinot noir wine in general showed the presence of beneficial genes for wine MLF (PAD, β-glucosidase, citrate lyase, PAP), while *O. oeni* isolates were especially lacking the PAD and PAP activities [53].

In the MLF of Patagonian Malbec wines, the content of the majority of endogenous alcohols and esters decreased with both *L. plantarum* and *O. oeni* [73]. However, increased formation of diethyl succinate (melon aroma) was detected in wine fermented with *O. oeni* or *L. plantarum* UNQLp155, but not in samples fermented with *L. plantarum* UNQLp11. Succinate was likely derived from partial TCA cycle of *L. plantarum*, subsequently esterified with ethanol at both COOH-groups by EstA or similar enzyme. Additionally, mixed culture fermentation (*L. plantarum* + *O. oeni*) led to increased formation of diethyl succinate compared to single strain fermentation. Release of β-citronellol in MLF by UNQLp11 and UNQOe73.2 was suggested be derived from β-glucosidase activity. A similar study setting was applied to Patagonian Pinot noir wines [184]. Most esters decreased in the Patagonian Pinot noir wine fermented with *L. plantarum* UNQLp11. However, *O. oeni* UNQOe 73.2 increased the content of various odorant ethyl esters, contributing to a notable change in the wine's volatile profile.

In the MLF of synthetic wine media, it was observed that *mle* expression in *L. plantarum* was induced at a lower pH (3.2 vs. 3.8) but reduced by increasing ethanol content [185]. In addition, the ability to tolerate combined stress during MLF (ethanol, low pH, malic acid) was higher in *L. plantarum* than in *O. oeni* when ethanol content was lower than 6 % (v/v). Co-inoculation of *S. cerevisiae* and *L. plantarum* also reduced the total fermentation time [164]. MLF with *L. plantarum* could therefore be more effective with co-inoculation of yeast rather than as a sequential fermentation after the primary fermentation.

2.8.3 Fruits and berries

2.8.3.1 Strain selection

While preservation of vegetables with spontaneous lactic acid fermentation has been utilized for several millennia, and is still in common practice today, berries and fruits have been traditionally preserved by jamming. In fact, bioprocessing of fruits and berries with lactic acid or MLF is a rather recent approach. In more recent studies, starter cultures were preferred over spontaneous fermentation, either as monocultures or as a mix of several strains or species, monocultures being the more common approach. A few strains that have been used extensively in a variety of materials include the type strain *L. plantarum* DSM 20174, the strain C2 isolated from carrots, and the strain POM1 isolated from tomatoes (**Table 7**). While the whole genome has been characterized for the strain WCFS1 [20], it has not been applied to food models.

Table 7. List of strains used in fermentations of various fruit materials, origin of strain, and materials it has been utilized in.

Strain code	***Strain origin***	***Used in material***
1MR20	Pineapple	Cherry juice [50,186], pineapple juice [50], cactus cladodes pulp [187]
CCM8	Cheese	Cherry juice [50,186], pineapple juice [50]
CIL6	Cherry	Cherry juice [50,186], pineapple juice [50], cactus cladodes pulp [187]
DC400	Sourdough	Cherry juice [50,186], pineapple juice [50]
DSM 20174	Pickled cabbage	Sea buckthorn [103,188,189], chokeberry [103], lingonberry [103], sea buckthorn / apple mix [188], pomegranate juice [190], noni juice [191]
1LE1	Pineapple	Elderberry juice [192–194], cherry juice [195]
1OR12	Pineapple	Pineapple juice [196]
285	Brazilian cheese	Elderberry juice [192–194], cherry juice [195]
90	Wine	Jujube juice [197], apple juice
AFI5	Apple	Apple by-product [198]
B42	Cheese	Orange juice [199]
B7	Sourdough	Bog bilberry juice [200]
BNCC 337796	Not reported	Blackberry juice [201], blueberry juice [201]
C1	Carrot	Elderberry juice, cherry juice [195]
C2	Carrot	*Myrtus communis* berries homogenate [202], pomegranate juice [5], cherry juice [50,186], pineapple juice [50]
C5	Carrot	Cherry juice [186]
C8-1	Pickles	Bog bilberry juice [200]
GIM1.140	Not reported	Papaya puree [203], mango slurry [204]

Strain code	***Strain origin***	***Used in material***
J26	Fermented dairy	Blueberry juice [205]
LP09	Commercial strain	Pomegranate juice [5]
Lp-115	Commercial strain	Mulberry juice [206], mixed juice (73% of acai berry, 17% of aronia, and 10% of cranberry) [207]
LS5	Not reported	Sweet lemon juice [208]
NCU116	Chinese sauerkraut	*Momordica charantia* juice [209]
POM1	Tomato	Elderberry juice [192–194], pomegranate juice [5], cherry juice [195], cactus cladodes pulp [187]
ST-III	Kimchi	Apple juice [210]
VTT E-78076	Beer	Lingonberry mash [211]
FP3	Sweet cherry	Sweet cherry [212]
KCTC 33131	Not reported	Cherry silverberry puree [213]
TMW 1.460	Spoiled beer	Cherry juice [186]

One approach for starter culture selection is to use an autochthonous culture, i.e. a starter culture isolated from the raw material [196,212,214]. The rationale for this approach is that the strain or isolate is adapted to the raw material, leading to an effective fermentation. A second approach is to use a strain with well-known or desired characteristics [197,199]. The third approach is to use a variety of cultures from different sources to screen for the strain with optimal properties [192]. The beneficial characteristics for wine starter culture are listed in **Section 2.12.2**, and these overlap to a certain degree with the desired properties for a strain to be used in fruit or berry fermentation. These properties include, but are not limited to: growth in and tolerance to low pH, tolerance to low temperature, ability to complete fermentation, presence of the malolactic gene, ability to tolerate and metabolize phenolic compounds, ability to synthesize antimicrobial compounds, activation of heterofermentative metabolism, ability to synthesize odor compounds or their precursors, lack of biogenic amine formation, ability to synthesize exo-polysaccharides, and an ability to increase antioxidant activity [21].

2.8.3.2 Fermentation trial set up

Lactic acid fermentation with *L. plantarum* has been applied to a variety of fruit and berry materials with cherry [195,212] and pomegranate juices [5,190,215] having been studied by multiple groups. Almost all of the studies retrieved have heat treatment prior to fermentation in order to remove the natural flora from the raw material (**Table 8**). Typically, the materials used were natural, meaning with little or no additional nutrients, and only in a few studies had the pH been significantly modified before fermentation. While in wine MLFs fermentation times of up to several weeks are used [73], in the fermentation of non-alcoholic fruits and berries short fermentation times are preferred, ranging from 24 to 336 hours with an average of 48 hours.

Table 8. Fermentation set-up in the studies related to the fermentation of fruit materials with *L. plantarum*.

Raw material	***Pretreatment***	***Strain(s) used***	***Ferm.***	***Storage***	***Ref.***
Apple by-product homogenate	HS 90 °C, 20 min.	AFI5 or AFI5 with *S. cerevisiae* AYI7	30°C, 72 h		[198]
Apple juice	None	ST-III	37 °C, 80 h	4 °C, 28 d	[210]
Apple juice, cv. Fuji	HS 72 °C, 15 min.	90	37°C, 48 h		[216]
Blackberry juice	HS 85 °C, 20 min.	BNCC 337796	37°C, 48 h		[201]
Blueberry (*Vaccinium ashei*) juice	HS 85 °C, 20 min.	BNCC 337796	37°C, 48 h		[201]
Blueberry juice	HS 85 °C, 15 min.	Four isolates	37 °C, 48 h		[217]
Blueberry juice	Pasteurization	J26	37°C, 24 h	4 °C, 28 d	[205]
Bog bilberry juice, pH 2.65 or 3.50	HS 85 °C, 15 min.	B7, C8-1	23°C, 336 h		[200]
Cactus cladodes pulp	None	CIL6, 1MR20, POM1	30 °C, 24 h		[187]
Carrot juice	HS 121 °C, 10 min. FS 0.22 µm	C2	30 °C, 24 h	4 °C, 21 d	[35]
Cherry juice	HS 121 °C, 10 min. FS 0.22 µm	C2, 1MR20, CIL6, POM1, DC400, CCM8	30 °C, 24 h	4 °C, 21 d	[50]
Cherry juice	HS 121 °C, 10 min.	C2, C5, 1MR20, CIL6, POM1, TMW 1.460	34 °C, 24 h		[186]
Cherry juice	Industrially pasteurized	POM1, C1, 1LE1, 285	30 °C, 48 h	4 °C, 12 d	[195]
Cherry silverberry puree	None	Mono- and mixed cultures of *L. plantarum* KCTC 33131 and *L. casei* KCTC 13086	35 °C, 72 h		[213]
Date fruit (cv. Siwi) homogenate	HS 90 °C, 20 min.	T1.3, T1.15, O1.8, T2.8, T1.18	30 °C, 60 h		[214]
Elderberry juice	Pasteurized	POM1, 1LE1, 285	30 °C, 48 h	4 °C, 12 d	[192]
Elderberry juice	Industrially pasteurized	POM1, C1, 1LE1, 285	30 °C, 48 h	4 °C, 12 d	[193,194]
Jujube juice cv. Muzao, cv. Hetian	HS 80 °C, 10 min.	90	37 °C, 48 h		[197]
Lingonberry mash	HS 80 °C, 5 min.	VTT E-78076	30°C, 72 h		[211]
Mango slurry	HS 90 °C, 10 min.	GIM1.140, *S. cerevisiae* DV10, or mixed	28 °C, 24 h		[204]

Raw material	***Pretreatment***	***Strain(s) used***	***Ferm.***	***Storage***	***Ref.***
Mix (acai berry (73%), aronia (17%), and cranberry (10%))	Not reported	Lp-115	37 °C, 36 h		207
Mix (cherries (26%, w/w), tomatoes (8%), blackberries (5%), prunes (31%))	HS 80 °C, 10 min.	Mix of *P. pentosaceus* SWE5, *L. plantarum* POM1 and Pr3, *W. cibaria* B6 and B1	25 °C, 24 h	4 °C, 30 d	218
Mix (kiwifruits (40%, w/w), fennels (7%), spinach (8%), and papaya (15%))	HS 80 °C, 10 min.	Mix of *L. plantarum* K3 and F6, *L. pentosus* P1, *W. cibaria* P9	25 °C, 24 h	4 °C, 30 d	218
Momordica charantia juice	HS 102 °C, 20 min.	NCU116	37 °C, 48 h		209
Mulberry juice	Ultrasonication	Lp-115	37 °C, 36 h		206
Myrtus communis homogenate	None	C2	30 °C, 48 h		202
Noni juice		DSM 20174	30°C, 72 h	4 °C, 28 d	191
Orange juice, cv. Tarocco and cv. Washington Navel	HS 80 °C, 40 sec.	B42	30 °C, 48 h		199
Papaya puree (45 %)	HS 90 °C, 10 min.	GIM1.140	37 °C, 48 h		203
Pineapple juice	HS 72 °C, 15 min.	1OR12	25 °C, 24 h	4 °C, 30 d	196
Pineapple juice	HS 121 °C, 10 min. FS 0.22 µm	C2, 1MR20, CIL6, POM1, DC400, CCM8	30 °C, 24 h	4 °C, 21 d	50
Pineapple juice	HS 121 °C, 10 min. FS 0.22 µm	C2	30 °C, 24 h	4 °C, 21 d	35
Pineapple juice	HS 121 °C, 10 min. FS 0.22 µm	Three isolates	30 °C, 24 h		219
Pomegranate juice	HS 80 °C, 5 min.	DSM 20174	30 °C, 72 h	4 °C, 28 d	220
Pomegranate juice	HS 80 °C, 5 min.	DSM 20174	30 °C, 72 h		190
Pomegranate juice (cv. Molfetta)	FS 0.22 µm	C2, POM1, LP09	30 °C, 120 h		5
Pomegranate juice (cv. Molfetta)	FS 0.22 µm	C2, POM1, LP09	30 °C, 120 h	4 °C, 30 d	221

Raw material	***Pretreatment***	***Strain(s) used***	***Ferm.***	***Storage***	***Ref.***
Sea buckthorn juice, cv. Jósef, mix (SB juice and apple juice, 1:1)	Pasteurization	DSM 16365, DSM 100813, DSM 13273, DSM 20174, DSM 10492, and DSM 6872	30°C, 72 h		[188]
Sweet cherry (*Prunus avium*) puree	HS 80 °C, 10 min.	Mix of *P. pentosaceus* SWE5 and *L. plantarum* FP3	25 °C, 36 h	4 °C, 60 d	[212]
Sweet lemon juice (cv. Jahrom)	HS 80 °C, 5 min.	LS5	37 °C, 48 h	4 °C, 28 d	[208]

Abbreviation: HS, heat sterilization; FS, filter sterilization; ferm., fermentation.

Many of the retrieved studies have included a storage trial to test cell viability during extended storage at +4 °C and monitor changes in chemical composition throughout the storage as well.

2.8.3.3 Changes in cell numbers, pH, carbohydrates, and organic acids

In the fermentation of berry or fruit materials with *L. plantarum*, inoculation levels varied from 4.5 to 8 log CFU/mL, with 7 log CFU/mL (**Table 9**) being the most common. The cell count increased in almost all instances during fermentation, typically 1–2 log CFU/mL, even in materials with an initial pH as low as 3. Storage trials showed that *L. plantarum* cell counts remain relatively stable for up to 60 days in storage at +4 °C. The only exception was made by [190] who reported that no bacteria was detected in pomegranate juice after 28 days of storage.

As expected, lactic and acetic acids increased in all materials after fermentation with *L. plantarum* (**Table 10**). Change in the content of individual sugars depended on the material, strain, and starter pH. For example, in the fermentation of orange juices, the sugar content was reduced in juices from cv. Washington Navel while no reduction was observed in juices from cv. Tarocco. The difference was associated with the ability to survive in the material, i.e., cell numbers increased in the former juice while they decreased in the latter. On the other hand, fermentation of cherry juice with the strain ILE led to an increase in the glucose and citric acid contents, while fermentation of the same juice with the strain C1 led to the opposite result. Related to juice pH, sugars were utilized more by the strains B8 and C8-1 in bog bilberry juice at pH 2.65 compared to the metabolic activity in juices a with pH of 3.50.

While MLF was observed in the majority of studies that measured changes in organic acid content, only a few studies reported significant increase in pH (0.5 units) (**Table 9**) [208,217]. Therefore, in most fruit or berry materials, MLF was either not intended to or is not appropriate for deacidification purposes.

2.8.3.4 Changes in volatile compound profiles

Depending on the strain or material, the ester content in the raw material is increased, not affected, or reduced (**Table 11**). By far the most common esters formed by *L. plantarum* in fruit materials are ethyl acetate and ethyl butyrate. Ethyl acetate is an expected volatile formed by *L. plantarum*, as both precursors, acetyl-CoA and ethanol, are derived from heterofermentative pathways of *L. plantarum*. Ester formation is not only related to the inherent properties of the starter culture, as high number of esters were generated by *L. plantarum* 90 in jujube juice from fruits belonging to cultivar Muzao, however, the same was not observed in juices made with the fruits of the cultivar Hetian [197]. The impact of pH on ester formation was observed in bog bilberry juice, as ester biosynthesis was activated at pH 2.65 while hydrolysis was more prevalent at pH 3.50 [200]. In

general, however, ester biosynthesis by *L. plantarum* is minor compared to commonly used yeasts, such as *S. cerevisiae* [204], and therefore modification of the aroma profile by *L. plantarum via* ester biosynthesis is not in general a viable approach. Unlike in wine MLF, fresh fruit material has a low number of precursor for ethyl ester biosynthesis (i.e., ethanol) which likely contributes to the reason why higher rate of ester biosynthesis being reported in general in wine MLF compared to fermentation of non-alcoholic material.

Increase in the content of volatile acids is often reported in fermented materials, as *L. plantarum* produces acetic acid, short-chain fatty acids, and various ketoacids in its metabolism. Additionally, as esters contribute to the fruity and floral notes in various berries and fruits [70], esterases produced by *L. plantarum* can hydrolyze these compounds to the corresponding alcohols and acids [80].

Common alcohols produced by *L. plantarum* during fermentation of fruit and berry materials include 3-methyl-1-butanol (fruity aroma) and 2-methyl-1-butanol (roasted aroma), derived from the metabolism of leucine and isoleucine, respectively. The floral aromatic alcohols phenylethyl alcohol and benzyl alcohol are commonly reported in materials fermented with *L. plantarum* as well. It can be speculated that these odor compounds are derived from the metabolism of phenylalanine, as phenylpyruvic acid is known to be oxidized to various odor compounds when exposed to the Mn^{2+} pool of *L. plantarum* [113,116] (**Section 2.6.2**). The other alcohols commonly present in fruit materials fermented with *L. plantarum* include 2-ethyl-1-hexanol (citrus), 1-hexanol (herbal), (*Z*)-3-hexen-1-ol (green), which are derived from fatty acid metabolism.

Aldehyde content often decreases during the fermentation of fruit materials and the compounds are either reduced to alcohols or oxidized to carboxylic acids. An excepetion to this was made by benzaldehyde (almond), the content of which was increased in fermented bog bilberry juice, pomegranate juice, and papay puree (**Table 11**). Similar to volatile phenolic alcohols, benzaldehyde is likely derived from phenylalanine metabolism [113,116]. Interestingly, a high content of 2-methylbenzaldehyde was formed in jujube juice when fermented with *L. plantarum* 90, which enhanced the cherry aroma of the material [197]. However, the precursor for this volatile has not been determined.

Effect of *L. plantarum* on aroma-active furans has been studied to a lesser extent. A decrease in furan content was reported in pomegranate juice [5], while an increase was reported in watermelon juice after fermentation [222].

While aldehydes are typically decreased in fermented fruit materials, the total ketone content is almost exclusively increased in the fermented fruit materials due to the formation of acetoin. As discussed earlier, in the heterofermentative pathway of *L. plantarum*, acetoin is the final downstream product as the species lacks enzymes to generate diacetyl or 2,3-butanediol.

Table 9. Change in the pH and cell number in various fruit materials fermented with *L. plantarum*. For the fermentation and storage conditions, see **Table 8**.

Raw material	***Strain used***	***pH***		***Cell number***			***Ref.***
		Start	Ferm.	Start	After ferm.	After stor.	
Apple by-product homogenate	AFI5 or AFI5 with *S. cerevisiae* AYI7	4.0	3.6	7 log cfu	8.9 log cfu		198
Apple juice	ST-III	6.0	3.7	6.3 log cfu	7.4 log cfu	6.5 log cfu	210
Apple juice, cv. Fuji	90	5.2	3.9	8 log cfu	12.8 log cfu		216
Blackberry juice	BNCC 337796	n.r.	n.r.	7.2 log cfu	8.4 log cfu		201
Blueberry (*Vaccinium ashei*) juice	BNCC 337796	n.r.	n.r.	7.2 log cfu	7.7 log cfu		201
Blueberry juice	Four isolates	4.0	4,5	7 log cfu	10 log cfu		217
Blueberry juice	J26	4.6	3.2	7.2 log cfu	9.1 log cfu	7.6 log cfu	205
Bog bilberry juice	B7, C8-1	2.7	2.6	8 log cfu			200
Bog bilberry juice	B7, C8-1	3.5	3.5	8 log cfu			200
Cactus cladodes pulp	CIL6, 1MR20, POM1	4.3	4.0	8 log cfu	9.5 log cfu		187
Carrot juice	C2	5.8	4.2	7 log cfu	9.2 log cfu	8.7 log cfu	35
Cherry juice	C2, 1MR20, CIL6, POM1, DC400, CCM8	4.0	4.0	7 log cfu	8.2 log cfu	7.5 log cfu	50
Cherry juice	C2, C5, 1MR20, CIL6, POM1, TMW 1.460	4.0	3.7	8 log cfu	9.1 log cfu		186
Cherry juice	POM1, C1, 1LE1, 285	3.6	3.6	7 log cfu	8.3 log cfu	8.4 log cfu	195
Cherry silverberry puree	Mono- and mixed cultures of *L. plantarum* KCTC 33131 and *L. casei* KCTC 13086	4.1	3.3	6 log cfu	9.5 log cfu		213
Date fruit (cv. Siwi) homogenate	T1.3, T1.15, O1.8, T2.8, T1.18	6.2	3.8	8 log cfu	9.1-9.5 log cfu		214
Elderberry juice	POM1, 1LE1, 285	n.r.	n.r.	7.1 log cfu	9.4 log cfu	9.3 log cfu	192
Elderberry juice	POM1, C1, 1LE1, 285	3.8	3.6	7.0 log cfu	9.5 log cfu	9.4 log cfu	193

(**Table 9** continued)

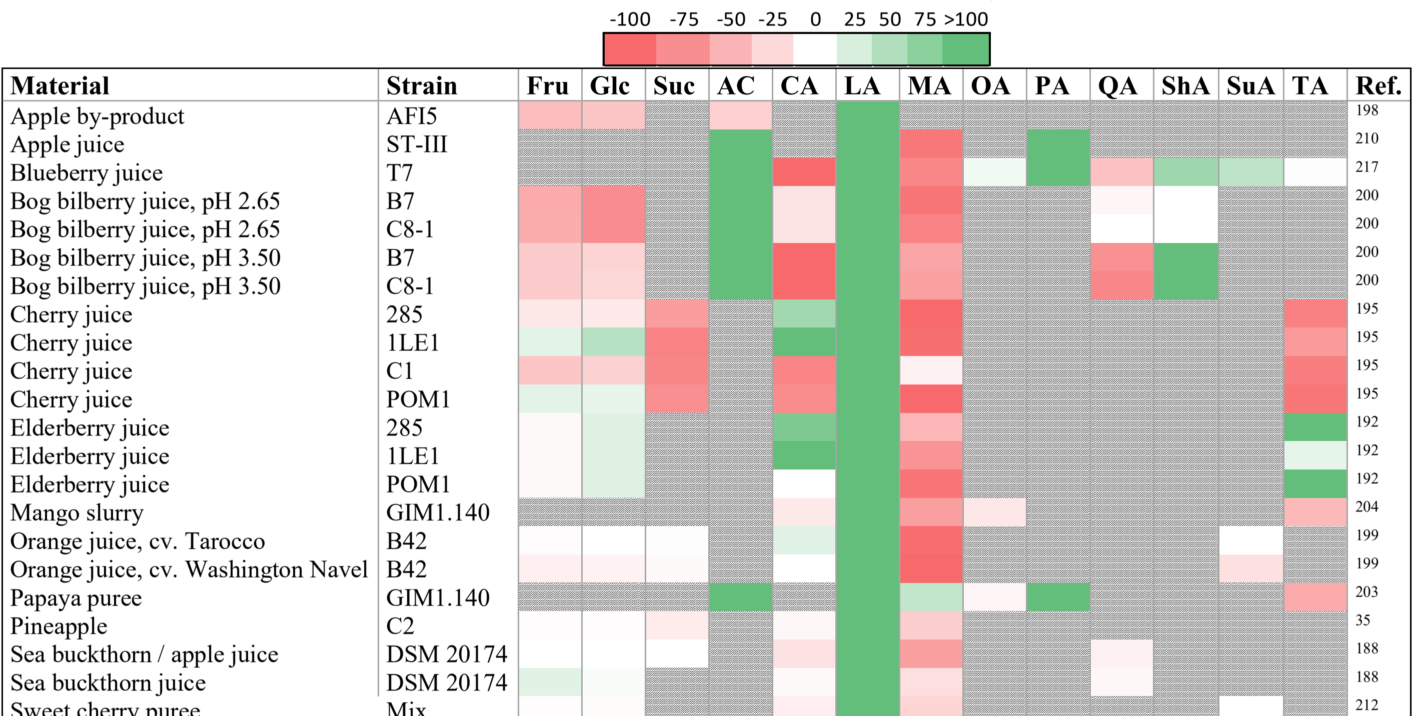
Elderberry juice	POM1, C1, 1LE1, 285	3.9	3.6	7 log cfu	9.3 log cfu	9.3 log cfu	194
Jujube juice cv. Muzao, cv. Hetian	90	5.0	n.r.	0.5%. v/v			197
Lingonberry mash	VTT E-78076	5.0	4.9	6 log cfu	6.7 log cfu		211
Mango slurry	GIM1.140, *S. cerevisiae* DV10, or mixed	4.1	3.5	7 log cfu	9 log cfu		204
Mix (acai berry (73%), aronia (17%), and cranberry (10%))	Lp-115	7	4.8	1 g/L			207
Mix (cherries (26%, w/w), tomatoes (8%), blackberries (5%), prunes (31%))	Mix of *P. pentosaceus* SWE5, *L. plantarum* POM1 and Pr3, *W. cibaria* B6 and B1	3.5	3.0	7 log cfu	9.5 log cfu	9.1 log cfu	218
Mix (kiwifruits (40%, w/w), fennels (7%), spinach (8%), and papaya (15%))	Mix of *L. plantarum* K3 and F6, *L. pentosus* P1, *W. cibaria* P9	4.0	3.2	7 log cfu	9.5 log cfu	9.0 log cfu	218
Momordica charantia juice	NCU116	4.6	3.3	7 log cfu	8 log cfu		209
Mulberry juice	Lp-115	n.r.	n.r.	1%. v/v			206
Myrtus communis homogenate	C2	5.1	3.4	7.5 log cfu			202
Noni juice	DSM 20174	4.9	4.0	4.5 log cfu	9.1 log cfu	5.3 log cfu	191
Orange juice, cv. Tarocco	B42	3.5	3.5	6 log cfu	7 log cfu		199
Orange juice, cv. Washington navel	B42	3.5	3.5	6 log cfu	8 log cfu		199
Papaya puree (45%)	GIM1.140	5.3	3.6	5 %			203
Pineapple juice	1OR12	3.5	2.7	7.0 log cfu	8.5 log cfu	7.3 log cfu	196
Pineapple juice	C2, 1MR20, CIL6, POM1, DC400, CCM8	3.6	3.4	7.0 log cfu	9.2 log cfu	0.2-0.8 red.	50
Pineapple juice	C2	3.7	3.3	7.0 log cfu	9.1 log cfu	8.4 log cfu	35
Pineapple juice	Three isolates	3.5		7 log cfu			219

(**Table 9** continued)

Pomegranate juice	DSM 20174	3.4	3,1	7 log cfu	8.5 log cfu	ND	220
Pomegranate juice	DSM 20174	3.1		7 log cfu	8.5 log cfu		190
Pomegranate juice (cv. Molfetta)	C2, POM1, LP09	n.r.	n.r.	7 log cfu			5
Pomegranate juice (cv. Molfetta)	C2, POM1, LP09	3.5	3.2	7 log cfu	9 log cfu	7 log cfu	221
SB juice and apple juice, 1:1	DSM 16365, DSM 100813, DSM 13273, DSM 20174, DSM 10492, and DSM 6872	3.2	3.30-3.32	1%. v/v			188
Sea buckthorn juice, cv. Józef	Same as above	3.0	3.0-3.1	1%. v/v			188
Sweet cherry (*Prunus avium*) puree	Mix of *P. pentosaceus* SWE5 and *L. plantarum* FP3	4.0	4.0	7 log cfu	9.5 log cfu	9 log cfu	212
Sweet lemon juice (cv. Jahrom)	LS5	3.4	3.8	7 log cfu	8.5 log cfu	7.1 log cfu	208

Abbreviations: n.r., not reported.

Table 10. Relative change (%) in the sugar and organic acid content in various fruit materials after fermentation with *L. plantarum*.



Material	Strain	Fru	Glc	Suc	AC	CA	LA	MA	OA	PA	QA	ShA	SuA	TA	Ref.
Apple by-product	AFI5														198
Apple juice	ST-III														210
Blueberry juice	T7														217
Bog bilberry juice, pH 2.65	B7														200
Bog bilberry juice, pH 2.65	C8-1														200
Bog bilberry juice, pH 3.50	B7														200
Bog bilberry juice, pH 3.50	C8-1														200
Cherry juice	285														195
Cherry juice	1LE1														195
Cherry juice	C1														195
Cherry juice	POM1														195
Elderberry juice	285														192
Elderberry juice	1LE1														192
Elderberry juice	POM1														192
Mango slurry	GIM1.140														204
Orange juice, cv. Tarocco	B42														199
Orange juice, cv. Washington Navel	B42														199
Papaya puree	GIM1.140														203
Pineapple	C2														35
Sea buckthorn / apple juice	DSM 20174														188
Sea buckthorn juice	DSM 20174														188
Sweet cherry puree	Mix														212

Abbreviations: Fru, fructose; glc, glucose; suc, sucrose; AC, acetic acid; CA, citric acid; LA, lactic acid; OA, oxalic acid; PA, pyruvic acid; QA, quinic acid; ShA, shikimic acid; SuA, succinic acid; TA, tartaric acid. Dashed lines mark that the compound was not detected or reported.

Table 11. Changes in the volatile profiles of various fruit materials fermented with *L. plantarum*. Compared to the fresh material, the green color marks an increase, the yellow color no significant change, and the red color a significant decrease in abundance. Dashed lines mark that the compound was not detected.

Material	Papaya puree (45%)[203]	Mango slurry[204]	Elder-berry[193]	Elder-berry[193]	Elder-berry[193]	Elder-berry[193]	Pome-granate juice[5]	Pome-granate juice[5]	Pome-granate juice[5]	Jujube juice, cv. Muzao[197]	Jujube juice, cv. Hetian[197]
Strain	GIM1.140	GIM1.140	POM1	1LE1	C1	285	C2	POM1	LP09	90	90
Total terpenes	–	green	green	green	green	green	yellow	green	green	–	–
2-Ethyl-1-hexanol	red	–	–	–	–	–	yellow	yellow	yellow	yellow	yellow
1-Hexanol	green	green	yellow	yellow	yellow	green	yellow	yellow	yellow	green	yellow
(*Z*)-3-Hexen-1-ol	–	green	yellow	yellow	yellow	green	yellow	yellow	yellow	–	–
3-Me-1-Butanol	green	yellow	green	green	green	red	green	green	green	red	red
2-Me-1-Butanol	–	–	–	–	–	–	green	green	green	green	green
Ethanol	green	green	green	green	green	green	yellow	yellow	yellow	red	red
Phenylethyl alcohol	green	green	green	green	green	green	–	–	–	–	–
Benzyl alcohol	green	green	–	–	–	–	–	–	–	yellow	yellow
Total alcohols	–	green	green	green	green	green	green	green	green	red	green
Ethyl acetate	green	red	green	green	green	red	yellow	yellow	yellow	green	yellow
Ethyl 3-methylbutanote	–	–	green	green	yellow	red	–	–	–	–	–
Ethyl butyrate	green	yellow	–	–	–	–	green	green	green	yellow	yellow
Ethyl propanoate	–	–	–	–	–	–	green	green	green	–	–
Ethyl octanoate	yellow	green	–	–	–	–	–	–	–	–	–
Ethyl decanoate	yellow	green	–	–	–	–	–	–	–	green	yellow

Material	Papaya puree (45%)[203]	Mango slurry[204]	Elder-berry[193]	Elder-berry[193]	Elder-berry[193]	Elder-berry[193]	Pome-granate juice[5]	Pome-granate juice[5]	Pome-granate juice[5]	Jujube juice, cv. Muzao[197]	Jujube juice, cv. Hetian[197]
Strain	GIM1.140	GIM1.140	POM1	1LE1	C1	285	C2	POM1	LP09	90	90
Ethyl hexanoate											
Isoamyl acetate											
Total esters											
Acetic acid											
3-Me-Butanoic acid											
Butanoic acid											
Octanoic acid											
Benzoic acid											
Total acids											
Acetaldehyde											
Benzaldehyde											
Nonanal											
Pentanal											
Hexanal											
Total aldehydes											
Acetoin											
Diacetyl											
2-Undecanone											
Total ketones											
4-Ethyl phenol											

Material	Mixed juice[207]	Cherry juice[195]	Cherry juice[195]	Cherry juice[195]	Cherry juice[195]	Bog bilberry juice, pH 2.65[200]	Bog bilberry juice, pH 2.65[200]	Bog bilberry juice, pH 3.50[200]	Bog bilberry juice, pH 3.50[200]	Lingon-berry puree[211]	Apple juice[216]
Strain	Lp-115	1LE1	285	C1	POM1	B7	C8-1	B7	C8-1	VTT E-78076	90
Total terpenes											
2-Ethyl-1-hexanol											
1-Hexanol											
(Z)-3-Hexen-1-ol											
3-Me-1-Butanol											
2-Me-1-Butanol											
Ethanol											
Phenylethyl alcohol											
Benzyl alcohol											
Total alcohols											
Ethyl acetate											
Ethyl 3-methylbutanote											
Ethyl butyrate											
Ethyl propinoate											
Ethyl octanoate											
Ethyl decanoate											
Ethyl hexanoate											
Isoamyl acetate											

Material	Mixed juice[207]	Cherry juice[195]	Cherry juice[195]	Cherry juice[195]	Cherry juice[195]	Bog bilberry juice, pH 2.65[200]	Bog bilberry juice, pH 2.65[200]	Bog bilberry juice, pH 3.50[200]	Bog bilberry juice, pH 3.50[200]	Lingon-berry puree[211]	Apple juice[216]
Strain	Lp-115	1LE1	285	C1	POM1	B7	C8-1	B7	C8-1	VTT E-78076	90
Total esters											
Acetic acid											
3-Me-Butanoic acid											
Butanoic acid											
Octanoic acid											
Benzoic acid											
Total acids											
Acetaldehyde											
Benzaldehyde											
Nonanal											
Pentanal											
Hexanal											
Total aldehydes											
Acetoin											
Diacetyl											
2-Undecanone											
Total ketones											
4-Ethyl phenol											

However, diacetyl is also formed in fermentations with *L. plantarum* due to the non-enzymatic oxidation of 2-acetolactate (**Fig. 3**). While moderate levels of diacetyl improve the sensory value of wines [223], it has not been established whether formation of acetoin is beneficial for the sensory value of non-alcoholic fruit materials.

While various authors promote the use of autochthonous cultures in plant-based fermentation [50,196,212], a case was made by Ricci et al. [193,195] to use dairy cultures in the fermentation of plant materials, as strains derived from fermented dairy produced more odor compounds compared to strains isolated from plant materials. This result was possibly due to the more complex metabolism of amino acids, as dairy strains have adapted to a protein rich environment. However, using this approach requires further studies to determine the suitability of dairy isolates for fermentation of fruit and berry materials.

The formation of volatile phenols in fermented fruit materials are discussed in the next section.

2.8.3.5 Biotransformation of phenolic compounds

While genomic studies have revealed the presence of a variety of mechanisms in *L. plantarum* related to phenolic metabolism, studies considering phenolic biotransformation in fruit and berry model foods provides highly varying and even often conflicting results (**Table 12**).

Studies have commonly focused on changes in phenolic acids during fermentation. Caffeic, *p*-coumaric and protocatechuic acids are almost exclusively metabolized, showing a decrease in concentration after fermentation. The main metabolites are dihydrocaffeic acid and catechol from caffeic acid and protocatechuic acid (PCA), respectively [186,195,209]. Formation of 4-ethyl phenol from *p*-coumaric acid by decarboxylation and reduction, respectively, was reported in elderberry [193] and cherry juices [195]. However, there was significant difference between the strains, and therefore, strain selection should consider the metabolic activity on *p*-coumaric acid as a factor to avoid formation of ethyl phenol, as this volatile is considered an off-odor [97]. Ricci et al. [193] also reported further metabolism of dihydrocaffeic acid to vinyl catechol.

Strain-dependent variation on phenolic acid metabolism was reported in the fermentation of cherry juice, as strain 1LE1 increased the content of PCA and caffeic acid, while strains 285 and POM1 decreased the content of these compounds [195].

Genomic studies have been unable to detect an effective enzyme from *L. plantarum* that effectively hydrolyzes chlorogenic acid (**Section 2.4.4**). It is possible that this gene is rare in *L. plantarum*, since only a few reports exist that show a significant decrease in chlorogenic acid content after fermentation with *L. plantarum* [200,201,213,217].

In **Section 2.6.5** it was discussed that phenylalanine is a potential precursor for various odor compounds. Another established pathway is metabolism into phenyllactic acid [146], however, only few studies report formation of this metabolite in fruit materials fermented with *L. plantarum* [194,195]. In addition, formation of *p*-OH-phenyllactic acid from tyrosine was reported in fermented cherry juice [195].

In the case of the metabolism of flavonols present in berries and fruits by *L. plantarum*, several glucosidases and rhamnosidases have been identified that can metabolize quercetin glycosides (**Section 2.5.3.1**). However, the author was unable to discover studies that show direct link between flavonol substrates and products during the fermentation of fruit materials. Increase in flavonol aglycones kaempferol and isorhamnetin were reported in cactus cladode pulps after fermentation [187]. Additionally, several reports have stated an increase in both flavonol glycoside and aglycone contents [195,206,217] while other studies report the opposite result [200,213]. The former result was possibly due to the release of cell-wall bound phenolic compounds either by endogenous or bacterial enzymatic activity [206].

Metabolism of proanthocyanidins and other condensed tannins by *L. plantarum* in fruit food models is poorly reported. Procyanidin dimers from cranberry (i.e., A2 and B2) were metabolized by *L. plantarum* ATCC BAA-793, yielding 3-(4-hydroxyphenyl)-propionic acid (phloretic acid) and 3-(3,4-hydroxyphenyl)-propionic acid [224]. Interestingly, the same study reported an improved utilization of oligosaccharides when *L. plantarum* was exposed to proanthocyanidins, showing a novel approach to improve fermentation in materials rich in fermentable fibers. Phloretic acid has also been reported as phenolic metabolite in fermented apple homogenate [198], bitter lemon juice [209], and cherry juice [186].

2.8.3.6 Changes in antioxidant capacity

Fermentation of plant materials with *L. plantarum* have often reported a beneficial impact on the antioxidant capacity, especially with the DPPH radical scavenging assay (**Table 13**). Typically, an increase in antioxidant activity has been associated with biomodification of phenolic compounds present in fruits and berries. The explanation is that the breakdown of phenolics introduces more hydroxyl groups that have antioxidant properties [4,187]. However, Hur et al. [4] discussed in their review that the overall picture is more complex (**Fig. 16**). For example, modification of redox balance plays a partial role, and that changes in pH during fermentation affects the deprotonation of polyphenols, which in turn affects the radical scavenging activity. Furthermore, while exopolysaccharides produced by *L. plantarum* have been shown chelate metal ions [225],

Table 12. Changes in the phenolic profiles of various fruit materials fermented with *L. plantarum*. Compared to the fresh material, the green color marks an increase, the yellow color no significant change, and the red color marks a significant decrease. Dashed lines mean that the compound was not detected.

Raw material	*Myrtus communis*[202]	Date fruit[214]	Apple by-product[198]	*Momordica charantia* juice[209]	Cherry juice[186]	Cherry juice[186]	Cherry juice[186]	Cherry juice[186]	Cherry juice[186]	Jujube juice, cv. Muzao[197]	Jujube juice, cv. Hetian[197]
Strain	C2	T1.3	AFI5	NCU116	C2	1MR20	CIL6	POM1	TMW 1.460	90	90
Gallic acid											
Protocatechuic acid											
p-Coumaric acid											
Vanillic acid											
Ferulic acid											
Syringic acid											
Caffeic acid											
Dihydrocaffeic acid											
Phloretic acid											
Vinyl phenol											
Cinnamic acid											
Benzoic acid											
Catechol											
Vinylcatechol											
Chlorogenic acid											
Ellagic acid											
Myricetin											
Quercetin											
Catechin											
Rutin											

Raw material	Mulberry juice[206]	Cherry silver-berry[213]	Cherry juice[195]	Cherry juice[195]	Cherry juice[195]	Cherry juice[195]	Elder-berry juice[194]	Elder-berry juice[194]	Elder-berry juice[194]	Elder-berry juice[194]	Apple juice[216]
Strain	Lp-115	KCTC 33131	1LE1	285	C1	POM1	1LE1	285	C1	POM1	90
Gallic acid											
Protocatechuic acid											
p-Coumaric acid											
Vanillic acid											
Ferulic acid											
Syringic acid											
Caffeic acid											
4-OH-Benzoic acid											
Dihydrocaffeic acid											
Cinnamic acid											
Benzoic acid											
Catechol											
Phenyllactic acid											
p-OH-Phenyllactic acid											
Chlorogenic acid											
Ellagic acid											
Quercetin											
Catechin											
Rutin											
Kaempferol											
Esculin											

Raw material	Bog bilberry juice, pH 2.65[200]	Bog bilberry juice, pH 2.65[200]	Bog bilberry juice, pH 3.50[200]	Bog bilberry juice, pH 3.50[200]	Lingon-berry puree[211]	Blue-berry juice[217]	Blue-berry juice[217]	Blue-berry juice[201]	Black-berry juice[201]
Strain	B7	C8-1	B7	C8-1	VTT E-78076	T7	T10	BNCC 337796	BNCC 337796
Gallic acid									
Protocatechuic acid									
p-Coumaric acid									
Ferulic acid									
Syringic acid									
Caffeic acid									
4-OH-Benzoic acid									
Chlorogenic acid									
Ellagic acid									
Myricetin									
Quercetin									
Catechin									
Rutin									
Shikimic acid									

Table 13. Change in the antioxidant capacity of various fruit materials after fermentation with *L. plantarum*.

Raw material	***Strain***	***DPPH****	***ABTS****	***FRAP****	***Other****	***Ref.***
Apple by-product homogenate	AFI5				Caco-2 cells: inhibited oxidative stress	198
Apple juice, cv. Fuji	90					216
Blackberry juice	BNCC 337796				Ferric reduction	201
Blueberry juice	BNCC 337796				Ferric reduction	201
Blueberry juice	J26				Caco-2 cells: inhibited oxidative stress	205
Blueberry juice	T7, T9, T10, T15				Ferric reduction	217
Cactus cladodes pulp	CIL6, 1MR20, POM1				Vitamin C retention	187
Cherry silverberry puree	KCTC 33131				Reducing power, SOD activity, H_2O_2 scavenging	213
Date fruit homogenate	T1.15, O1.8, T2.8, T1.18					214
Jujube juice	90					197
Mango slurry	GIM1.140					204
Mix (acai berry (73%), aronia (17%), and cranberry (10%))	Lp-115				ORAC	207
Momordica charantia juice	NCU116					209
Mulberry juice	Lp-115				RP-CA	206
Myrtus communis homogenate	C2				Inhibition of lipid peroxidation	202
Noni juice	BCRC 10069				Reducing power	191
Papaya puree (45%)	GIM1.140				CUPRAC	203
Pineapple juice	1OR12					196
Pomegranate juice	DSM 20174					190
Sea buckthorn juice	DSM 10492				ORAC	188
Sea buckthorn juice	DSM 20174				ORAC	188

Raw material	***Strain***	***DPPH****	***ABTS****	***FRAP****	***Other****	***Ref.***
Sweet cherry (*Prunus avium*) puree	Mix of *P. pentosaceus* SWE5 and *L. plantarum* FP3					212
Sweet lemon juice	LS5				Vitamin C retention	208

* The green color marks a significant increase and the red color marks a significant decrease in the antioxidant capacity after fermentation. The yellow color marks that the change during fermentation was not statistically significant ($p > 0.05$). Dashed filling means that the assay was not studied.

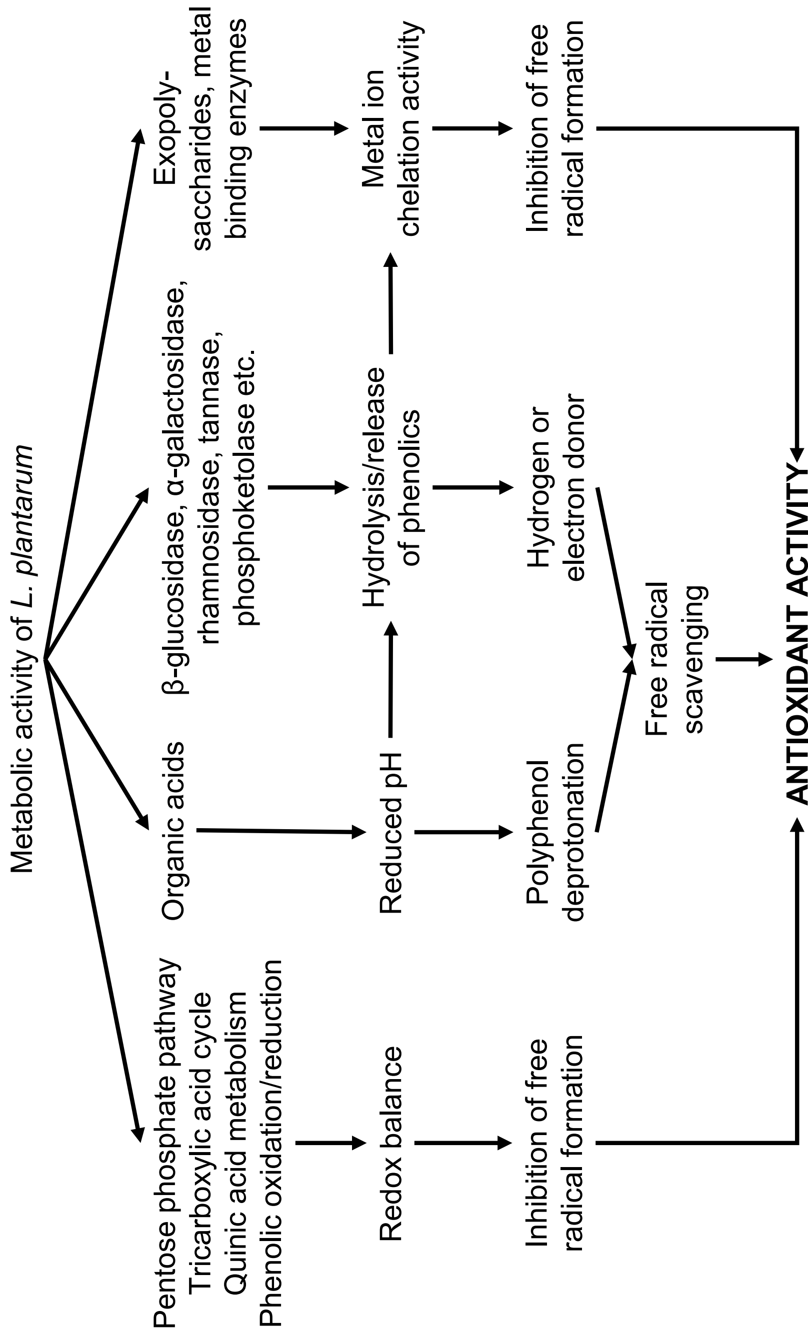


Fig. 16. Factors that contribute to the change in the antioxidant activity during fermentation of plant materials with *L. plantarum*. Adapted from [4].

metal chelating activity is not often beneficially impacted in the fermentation of fruit materials, since the FRAP results in most studies remain unaffected [203] or are even reduced after fermentation [209] (**Table 13**).

Several studies have reported a higher content of ascorbic acid in inoculated fruit materials compared to a control without bacteria after the incubation period [187,208]. While no direct link has been established, it can be speculated that increase in antioxidant capacity during fermentation protected the ascorbic acid from oxidation. It was shown by Hashemi et al. that the effect was also apparent during storage, and after 28 days at +4 °C fermented lemon juice had a higher ascorbic acid content compared to non-fermented juice, even when the non-fermented fresh juice initially had a higher concentration [208]. Other groups of antioxidant compounds affected by processing and extended storage are anthocyanins and carotenoids, and protection of these compounds from degradation by isolate *L. plantarum* T10 was reported in blueberry juice [217] and by the strain 1MR20 in cactus cladode pulp [187], respectively. The ability to impact antioxidant activity and to protect ascorbic acid or anthocyanins from degradation is a strain-dependent property. For example, in the fermentation of sea buckthorn juice, the strain DSM 20174 significantly increased the ORAC value while the strain DSM 10492 showed the opposite effect [188].

2.9 Compositional properties of berries

2.9.1 Sugar, sugar alcohols, and organic acids in commercially important berries

The main sugars in most common berries are glucose, fructose, and sucrose. Sweet sugar alcohol sorbitol has been detected in substantial amounts in chokeberry, rowanberry, sweet cherry, and eastern shadbush (**Table 14**). Citric acid and malic acid are the main acids in the majority of the commonly cultivated or wild berries used by the food industry. In addition, some species also produce substantial amounts of quinic acid (e.g. sea buckthorn) or tartaric acid (grapevine, cranberry, bilberry).

High amounts of citric acid (> 10 g/kg FW) have been reported in raspberries, lingonberries, jostaberries, dog rose hips, and gooseberries. Various currants (black, white, red) contain especially high amounts of citric acid, up to 24 g/kg FW. Berry species that have shown a high malic acid content (> 10 g/L) include rowanberry, sour cherry, chokeberry, hardy kiwifruit, jostaberry, sea buckthorn, red gooseberry, and cranberry (**Table 14**) [226–228]. Especially high content of L-malic acid has been detected in sour cherry with up to 20 g/L of malic acid reported, and in rowanberry and sea buckthorn,

Table 14. Sugar, organic acid, pH, and sugar/acid ratio in various berries. The concentrations are g/L or g/kg FW expect mg/kg FW for shikimic acid.

Species	***Type***	***Location***	***pH***	***Fructose***	***Glucose***	***Sucrose***	***Sorbitol***	***S/A ratio****	***Ref.***
American cranberry	Cultivated	Slovenia		53.7 ± 1.3	55 ± 1.2	6.5 ± 0.2	n.d.	6.8 ± 0.6	226
Bilberry	Wild	Finland	2.98 ± 0.03	40.9 ± 0.2	31.5 ± 0.3	0.6 ± 0.0		5.8	229
Bilberry	Wild	Slovenia		22.4 ± 2.1	20.5 ± 1.9	0.3 ± 0.1	n.d.	3.9 ± 0.4	226
Black crowberry	Wild	Finland	3.52 ± 0.03	18.0 ± 0.1	24.8 ± 0.1	0.4 ± 0.0		6.5	229
Black currant	Cultivated	Slovenia		19.2 ± 0.2	13.6 ± 0	10.3 ± 1.1	n.d.	1.7 ± 0.3	226
Black mulberry	Cultivated	Slovenia		39.9 ± 4.7	36.8 ± 4.8	n.d.	n.d.	13.6 ± 2.3	226
Blackberry	Cultivated	Slovenia		26.9 ± 1.3	26.7 ± 1.3	1.2 ± 0.0	n.d.	6.8 ± 0.8	226
Blackberry	Wild	Slovenia		35.4 ± 2.8	35.3 ± 2.8	1.3 ± 0.1	n.d.	12.9 ± 2.2	226
Blackcurrant	Cultivated	Finland	3.04 ± 0.03	36.2 ± 0.1	31.9 ± 0.3	2.1 ± 0.0		2.6	229
Chokeberry	Cultivated	Slovenia		28.2 ± 1.2	35.4 ± 2.7	4.1 ± 1.7	46.2 ± 4.9	6.3 ± 0.6	226
Cloudberry	Wild	Finland	3.20 ± 0.03	27.0 ± 0.0	25.2 ± 0.0	n.d.		4.4	229
Cranberry	Wild	Finland	2.37 ± 0.03	20.7 ± 0.0	27.8 ± 0.1	0.2 ± 0.0		1.6	229
Dog rose	Wild	Slovenia		68.1 ± 2.2	89.6 ± 3.3	6.4 ± 0.5	n.d.	8.5 ± 0.8	226
Eastern shadbush	Cultivated	Slovenia		53.5 ± 3.0	50.6 ± 2.9	1.2 ± 0.1	53.3 ± 3.3	14.4 ± 0.9	226
Elderberry	Wild	Slovenia		27.8 ± 1.3	26.1 ± 2.1	1.5 ± 0.2	n.d.	4.6 ± 0.2	226
Goji berry	Cultivated	Slovenia		23.1 ± 3.7	23.1 ± 3.5	2.7 ± 0.4	n.d.	16.3 ± 3.3	226
Grapevine	cv. Beyaz Kismis	Turkey		155.5 ± 4	164.7 ± 2,1	n.d.	n.d.	4.4	230
Grapevine	cv. Kirmizi Kismis	Turkey		93.3 ± 1,6	104.9 ± 1,3	n.d.	n.d.	1.3	230
Hardy kiwifruit	Cultivated	Slovenia		27.1 ± 2.4	20.5 ± 3.4	100.5 ± 11.2	n.d.	4.6 ± 0.6	226
Highbush blueberry	Cultivated	Slovenia		39.3 ± 1.8	38.6 ± 1.7	0.2 ± 0.0	n.d.	9.3 ± 2.3	226
Japanese wineberry	Cultivated	Slovenia		26.2 ± 1.0	27.7 ± 0.9	0.1 ± 0.0	n.d.	6.5 ± 1	226
Jostaberry	Cultivated	Slovenia		49.4 ± 2.0	38.9 ± 1.8	7.7 ± 0.4	n.d.	2.9 ± 0.1	226
Lingonberry	Wild	Finland	2.67 ± 0.03	42.3 ± 0.3	42.4 ± 0.4	1.2 ± 0.0		3.7	229
Lingonberry	Wild	Slovenia		29.2 ± 0.7	37.9 ± 1.3	4.1 ± 0.5	n.d.	2.7 ± 0.2	226

Species	*Type*	*Location*	*pH*	*Fructose*	*Glucose*	*Sucrose*	*Sorbitol*	*S/A ratio**	*Ref.*
Raspberry	Cultivated	Slovenia		24.2 ± 2.1	21.2 ± 1.8	n.d.	n.d.	5.2 ± 1.3	226
Raspberry	Wild	Slovenia		25.9 ± 2.0	24.6 ± 1.8	0.5 ± 0.1	n.d.	3.9 ± 0.4	226
Red currant	Cultivated	Slovenia		20.8 ± 0.6	16.8 ± 0.4	0.5 ± 0.1	n.d.	2.1 ± 0.1	226
Red gooseberry	Cultivated	Finland	2.96 ± 0.03	46.7 ± 0.3	44.3 ± 0.3	5.1 ± 0.0		4.4	229
Red gooseberry	Cultivated	Slovenia		33.8 ± 3.2	33.2 ± 2.8	15.1 ± 2.2	n.d.	2.9 ± 0.2	226
Red raspberry	Wild	Finland	3.28 ± 0.03	57.5 ± 0.1	47.2 ± 0.1	0.5 ± 0.0		5.8	229
Redcurrant	Cultivated	Finland	2.91 ± 0.03	24.2 ± 0.2	22.2 ± 0.3	n.d.		1.6	229
Rowanberry	Wild	Slovenia		24.8 ± 1.7	52.9 ± 3.2	5.2 ± 1.5	134.1 ± 3.3	5.1 ± 0.4	226
Sea buckthorn	cv. Avgustinka	Finland	2.66 ± 0.13	4.1 ± 1.3	20.9 ± 1.9	n.d.	n.d.	0.6	228
Sea buckthorn	cv. Botanicheskaya	Finland	2.57 ± 0.01	3.4 ± 1.3	21.4 ± 4.3	n.d.	n.d.	0.6	228
Sea buckthorn	cv. Chuiskaya	Canada	2.67 ± 0.05	26.2 ± 2.5	23.3 ± 4.3	0.6 ± 0.5	n.d.	1.8	228
Sea buckthorn	cv. Oranzhevaya	Canada	2.82 ± 0.05	7.5 ± 1.5	17.5 ± 5.6	n.d.	n.d.	0.8	228
Sea buckthorn	cv. Pertsik	Finland	2.60 ± 0.01	7.0 ± 3.6	31.3 ± 6.9	n.d.	n.d.	0.8	228
Sea buckthorn	cv. Prozcharachnaya	Finland	2.67 ± 0.07	4.6 ± 0.9	20.5 ± 5.1	0.1 ± 0.1	n.d.	0.7	228
Sea buckthorn	cv. Trofimovskaya	Finland	2.60 ± 0.01	7.6 ± 1.7	29.0 ± 4.2	n.d.	n.d.	1.0	228
Sea buckthorn	cv. Vitaminaya	Canada	3.05 ± 0.03	10.8 ± 1.8	35.0 ± 4.6	0.5 ± 0.4	n.d.	1.6	228
Sour cherry	cv. Montmorency	Poland		4.2 ± 0.1	3.3 ± 0.1	n.d.	2.8 ± 0.1	0.8	231
Sour cherry	cv. Paraszt Meggy	Poland		4.1 ± 0.1	4.7 ± 0.1	n.d.	2.9 ± 0.0	0.5	231
Strawberry	Cultivated	Finland	3.50 ± 0.03	34.4 ± 1.7	33.1 ± 1.7	0.6 ± 0.0		6.1	229
Strawberry	Cultivated	Slovenia		30.2 ± 1.4	27.3 ± 1.0	2.5 ± 0.8	n.d.	5.7 ± 0.4	226
Sweet cherry	cv. Fercer	Slovenia		64.2 ± 5.1	84.4 ± 5.7	9.4 ± 0.6	18.9 ± 1.3	20.4	232
Sweet cherry	cv. Lapins	Slovenia		79.9 ± 3.4	93.7 ± 3.1	7.5 ± 0.3	14.4 ± 0.3	52.8	232
White currant	Cultivated	Slovenia		20.0 ± 0.9	19.1 ± 1.1	6.0 ± 0.7	n.d.	2.2 ± 0.1	226
White gooseberry	Cultivated	Slovenia		18.7 ± 2.1	17.5 ± 3.4	2.1 ± 0.4	n.d.	1.6 ± 0.3	226
Whitecurrant	Cultivated	Finland	3.04 ± 0.03	48.1 ± 0.3	50.0 ± 0.3	n.d.		3.5	229
Wild strawberry	Wild	Slovenia		21.3 ± 1.8	19.7 ± 1.4	13.6 ± 1.9	n.d.	3.5 ± 0.4	226

Species	*Type*	*Location*	*Benzoic acid*	*Citric acid*	*Malic acid*	*Quinic acid*	*Shikimic acid*	*Tartaric acid*	*Ref.*
American cranberry	Cultivated	Slovenia		14.7 ± 0.9	0.7 ± 0.2		17.7 ± 0.7	2.0 ± 0.1	226
Bilberry	Wild	Finland	n.d.	8.4 ± 0.2	4.2 ± 0.1				229
Bilberry	Wild	Slovenia		5.7 ± 0.3	2.7 ± 0.2		71.3 ± 4.3	1.9 ± 0.0	226
Black crowberry	Wild	Finland	0.1 ± 0.0	2.3 ± 0.3	4.3 ± 0.2				229
Black currant	Cultivated	Finland	n.d.	23.8 ± 0.3	3.5 ± 0.1				229
Black currant	Cultivated	Slovenia		12.6 ± 1.9	7.3 ± 1.1		26.5 ± 4.4	1.4 ± 0.1	226
Black mulberry	Cultivated	Slovenia		4.5 ± 0.4	0.7 ± 0.1		13.6 ± 1.0	n.d.	226
Blackberry	Cultivated	Slovenia		5.6 ± 0.4	2.1 ± 0.2		28.2 ± 2.5	n.d.	226
Blackberry	Wild	Slovenia		4.1 ± 0.6	1.1 ± 0.1		89.3 ± 3.8	n.d.	226
Chokeberry	Cultivated	Slovenia		1.3 ± 0.2	12.2 ± 0.2		55.4 ± 1.2	0.1 ± 0.0	226
Cloudberry	Wild	Finland	0.5 ± 0.1	3.7 ± 0.0	7.7 ± 0.0				229
Cranberry	Wild	Finland	0.2 ± 0.0	14.8 ± 0.5	16.2 ± 0.4				229
Dog rose	Wild	Slovenia		12.8 ± 1.4	5.3 ± 0.3		19.5 ± 2.6	n.d.	226
Eastern shadbush	Cultivated	Slovenia		0.9 ± 0.0	7.4 ± 0.2		74.2 ± 2.8	0.1 ± 0.0	226
Elderberry	Wild	Slovenia		9.4 ± 0.4	1.7 ± 0.2		79 ± 22.3	0.4 ± 0.0	226
Goji berry	Cultivated	Slovenia		2.1 ± 0.3	1.4 ± 0.1		27.5 ± 3.7	n.d.	226
Grapevine	cv. Beyaz Kismis	Turkey		0.3 ± 0.0	1.8 ± 0.1		n.d.	4.9 ± 0.2	230
Grapevine	cv. Kirmizi Kismis	Turkey		0.3 ± 0.0	1.5 ± 0.2		n.d.	12.7 ± 0.1	230
Hardy kiwifruit	Cultivated	Slovenia		5.9 ± 0.4	13.3 ± 0.9		9.3 ± 1.6	n.d.	226
Highbush blueberry	Cultivated	Slovenia		10.3 ± 1.5	0.6 ± 0.1		27.2 ± 3.8	n.d.	226
Japanese wineberry	Cultivated	Slovenia		7.3 ± 0.4	1.6 ± 0.2		1.0 ± 0.1	0.1 ± 0.0	226
Jostaberry	Cultivated	Slovenia		15.3 ± 0.4	12.1 ± 0.4		843.7 ± 40	0.2 ± 0.0	226
Lingonberry	Wild	Finland	0.7 ± 0.1	18.2 ± 0.7	4.2 ± 0.5				229
Lingonberry	Wild	Slovenia		20.0 ± 1.4	2.3 ± 0.1		40.9 ± 8.1	3.4 ± 0.4	226
Raspberry	Cultivated	Slovenia		10.8 ± 0.6	0.9 ± 0.1		14.2 ± 1.4	0.1 ± 0.0	226
Raspberry	Wild	Slovenia		11.4 ± 0.2	1.8 ± 0.1		18.9 ± 1.3	n.d.	226

Species	***Type***	***Location***	***Benzoic acid***	***Citric acid***	***Malic acid***	***Quinic acid***	***Shikimic acid***	***Tartaric acid***	***Ref.***
Red currant	Cultivated	Slovenia		14.7 ± 0.6	2.6 ± 0.1		352.5 ± 9.7	0.4 ± 0.0	226
Red gooseberry	Cultivated	Finland	n.d.	11.1 ± 0.1	10.8 ± 0.2				229
Red gooseberry	Cultivated	Slovenia		8.6 ± 0.4	11.6 ± 0.8		722.3 ± 11.3	0.1 ± 0.0	226
Red raspberry	Wild	Finland	n.d.	15.2 ± 0.3	2.9 ± 0.1				229
Redcurrant	Cultivated	Finland	n.d.	24.7 ± 0.8	4.9 ± 0.3				229
Rowanberry	Wild	Slovenia		1.2 ± 0.1	30.3 ± 0.9		16.1 ± 1	0.4 ± 0.0	226
Sea buckthorn	cv. Avgustinka	Finland		0.5 ± 0.2	32.0 ± 3.7	13.0 ± 0.6		n.d.	228
Sea buckthorn	cv. Botanicheskaya	Finland		0.2 ± 0.1	28.9 ± 3.1	16.5 ± 2.3		n.d.	228
Sea buckthorn	cv. Chuiskaya	Canada		0.3 ± 0.1	13.4 ± 1.9	14.5 ± 2.5		n.d.	228
Sea buckthorn	cv. Oranzhevaya	Canada		0.5 ± 0.1	19.9 ± 1.3	12.8 ± 1.2		n.d.	228
Sea buckthorn	cv. Pertsik	Finland		0.3 ± 0.0	32.8 ± 0.5	15.5 ± 4		n.d.	228
Sea buckthorn	cv. Prozcharachnaya	Finland		0.4 ± 0.1	29.0 ± 2.9	13.9 ± 2.7		n.d.	228
Sea buckthorn	cv. Trofimovskaya	Finland		0.3 ± 0.2	28.2 ± 1.4	12.3 ± 1.6		n.d.	228
Sea buckthorn	cv. Vitaminaya	Canada		0.2 ± 0.0	8.4 ± 0.7	20.5 ± 1.2		n.d.	228
Sour cherry	cv. Montmorency	Poland			10.3 ± 0.0		16.7 ± 0.4		231
Sour cherry	cv. Paraszt Meggy	Poland			19.6 ± 0.0		38.6 ± 0.7		231
Strawberry	Cultivated	Finland	n.d.	6.8 ± 0.3	4.5 ± 0.2				229
Strawberry	Cultivated	Slovenia		9.3 ± 0.4	1.0 ± 0.2		7.9 ± 0.4	n.d.	226
Sweet cherry	cv. Fercer	Slovenia		0.5 ± 0.1	8.1 ± 1.0		26.7 ± 1.3		232
Sweet cherry	cv. Lapins	Slovenia		0.2 ± 0.0	3.5 ± 0.2		8.4 ± 0.2		232
White currant	Cultivated	Slovenia		17.4 ± 0.6	2.0 ± 0.1		280.6 ± 7.9	0.2 ± 0.0	226
White gooseberry	Cultivated	Slovenia		10.3 ± 0.7	8.0 ± 0.3		580.1 ± 23.3	0.2 ± 0.0	226
Whitecurrant	Cultivated	Finland	n.d.	24.0 ± 0.5	4.1 ± 0.1				229
Wild strawberry	Wild	Slovenia		11.7 ± 1.4	2.0 ± 0.2		8.9 ± 0.9	0.1 ± 0.0	226

Abbreviations: S/A ratio, sugar/acid ratio

with concentrations over 30 g/L FW being reported in berry juices. Benzoic acid present in cranberry and cloudberry potentially limits the utilization of MLF at a low pH [229].

In addition to berries, apples, regardless of the variety, have consistently high L-malic acid content (4.40–9.50 g/L) [233]. On the other hand, genotypical difference in L-malic acid content in apricot varieties grown in China was nearly 10-fold (1.89 and 12.8 g/kg in fruits of 'Katy' and 'Xingmei', respectively) [234]. Therefore, when selecting material for MLF, variation in the sugar and acid content dependent on the subspecies, therefore the cultivar and growth location needs be taken into consideration.

For effective MLF to be initiated by *L. plantarum*, a minimum concentration of 5 mM of L-malic acid was required, corresponding to 0.67 g/L [63]. This means that with few exceptions almost all common cultivated and wild berry species would have the necessary L-malic acid content for MLF (**Table 14**) [226–228]. However, to maintain the MLF for an extended period, the L-malic content should be significantly higher.

2.9.2 Free amino acid content of sea buckthorn, chokeberry, and lingonberry

Typically *L. plantarum* lacks the ability to cleave large polypeptides [20]. Therefore, the readily available nitrogen sources in berry fermentation would be the free amino acids. The content of glutamate, branched-chain amino acids (Val, Ile, Leu), and arginine, are especially important, as the absence of any of the previously mentioned typically halts growth of *L. plantarum*. In addition, growth is reduced in the absence methionine, glycine, phenylalanine, and tryptophan [110,111].

In general, the free amino acid composition of berries varies and is dependent on the genotype and growth conditions (**Fig. 17**). In sea buckthorn as well as in lingonberry, aspartaric acid or asparagine are the most abundant amino acids, and can be up to 85% of the total amino acid content [235,236]. A high relative content of proline has also been detected in sea buckthorn [10]. In sea buckthorn berries, general lack of glutamic acid is a potential limitation in fermentation. However, the glutamic acid precursor 2-oxoglutaric acid has been reported from sea buckthorn berries (unpublished data of this thesis work). Lingonberries are rich in arginine, but have a low content of leucine and isoleucine, while chokeberries lack tryptophan [236]. Compared to the two previously mentioned berries, chokeberries have a more diverse amino acid profile, glutamic acid being present in high amounts in berries from both Poland and South Korea [237,238]. All three berry species have either a low or no content of methionine.

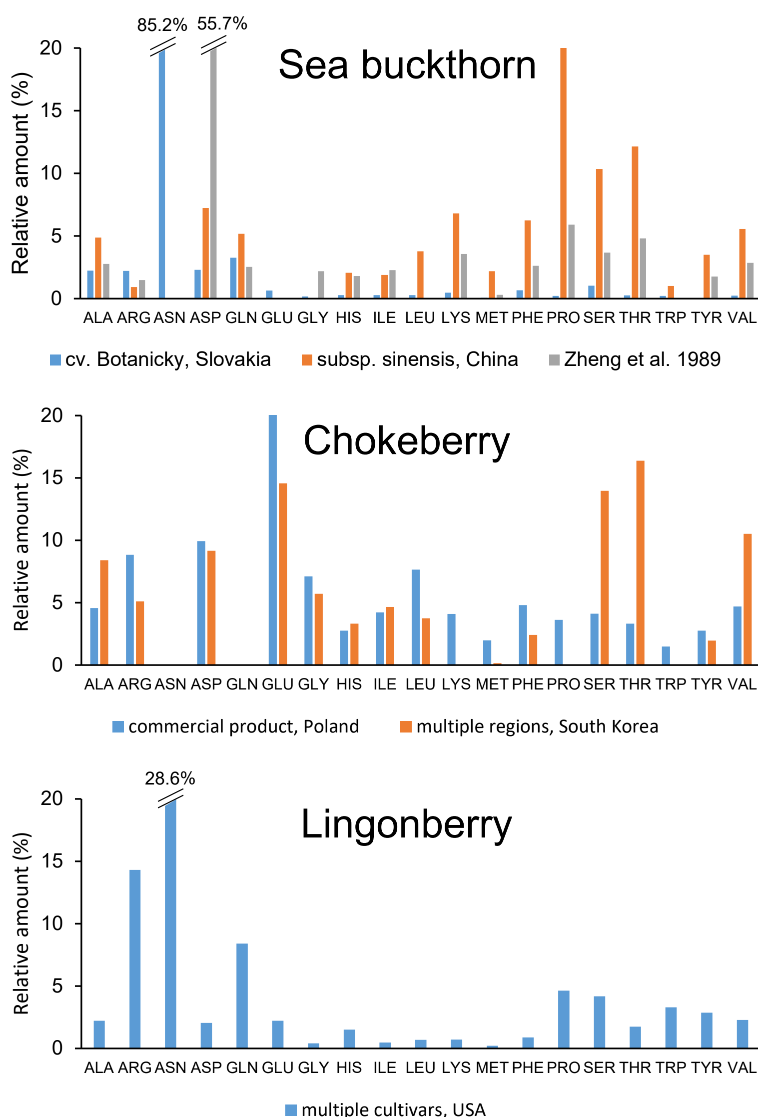


Fig. 17. Reported free amino acid profiles (relative abundance (%) out of total amino acids, w/w) berries of sea buckthorn [10,235], *Aronia melanocarpa* [237,238], and lingonberry [236] (average of multiple cultivars).

2.9.3 Phenolic content of sea buckthorn, chokeberry, and lingonberry

Phenolic compounds are a complex class of plant secondary metabolites that protect plants from various biotic (e.g., infection) and abiotic (photo-oxidation) stresses. Earlier, phenolic compounds were considered as anti-nutrients, as they inhibit various enzymes relevant to metabolism in mammals and reduce the bioavailability of iron and zinc. However, it has subsequently been discovered

that phenolic compounds possess various benefits to health due to the strong antioxidant activity, such as antidiabetic activity [239]. Recently, plant phenolics have been shown to promote the growth of the loosely defined beneficial gut microbiota [240]. In berries, the important subclasses of phenolics are flavonols, flavan-3-ols, hydrolyzable tannins, phenolic acids, and anthocyanins [241]. In addition to biological activities, many phenolic compounds contribute to the sensory quality of berries being bitter and/or astringent compounds, depending on the structure [242]. Numerous substitutions or ester formations with various sugars and acyl groups mean that the profile of phenolic compounds in fruits and berries are often a very complex mixture. However, extraction with various organic solvents, fractionation of crude extracts with HPLC or column chromatography, followed by structural elucidation with LC-MS and NMR have allowed identification and quantification of phenolic compounds in berry and fruit materials (**Table 15**) [243,244].

2.9.3.1 Phenolic acid content in sea buckthorn berry

Only a few reports exist that describe the phenolic acid content in sea buckthorn berries in detail [245–247] together with several sources that include limited reporting [103,244] (**Table 17**). In berries belonging to the subspecies *turkestanica*, 57% the phenolic acids were present as glycosides [245], while phenolic acid esters accounted for over 50 % of the total in several cultivars belonging to ssp. *mongolica* [246]. In turn, free phenolic acids accounted for 20% but only around 2% of total acids in [245] and [246], respectively. The main phenolic acids are typically hydroxybenzoic acids, which are the derivates of gallic acid and protocatehuic acid [103,245,246,248]. However, in one instance, *p*-coumaric acid was defined as the main phenolic acid in berries grown both in Sweden and India [247]. Additionally, a high content of salicylic acid was reported in multiple cultivars in berries grown in Poland and Belarus [246].

2.9.3.2 Flavonol content of chokeberry and lingonberry

In black chokeberries, flavonols are the fourth most abundant phenolic subclass, with concentrations ranging between 0.19-0.58 mg/g FW (**Table 18**). In *Aronia* spp., hyperoside (quercetin-3-*O*-galactoside), rutin (quercetin-3-*O*-rutinoside), and isoquercetin (quercetin-3-*O*-glucoside) are typically reported as the most abundant flavonol glycosides, making up to 90 % of total flavonols. While the profile varies depending on the species and the cultivar, quercetin-3-*O*-galactoside is, in general, the most abundant flavonol glycoside. Other quercetin derivates detected in black chokeberry are 3-*O*-vicianoside and 3-robinobioside [249,250]. In addition, isorhamnetin derivates with unidentified sugar moieties have been identified [250].

Table 15. Details of the raw materials, extraction solvent and analyzed flavonoid subclasses in selected studies of phenolic content of chokeberry (CB), lingonberry (LB) and sea buckthorn (SB) berries.

Berry	***(Sub)species***	***Cultivar/wild***	***Growth location***	***A***	***F***	***PA***	***PAC***	***Extraction***	***Ref.***
CB	*A. arbutifolia*	3 biotypes	USA	x	x	x	x	70/30/0.5% acetone/water/acetic acid	251
CB	*A. melanocarpa*	wild	Canada	x	x	x		80% acidic acetone	252
CB	*A. melanocarpa*	Wild and 3 cultivars	Croatia	x	x	x		Acidic ethanol	253
CB	*A. melanocarpa*		Norway	x	x	x		Acidic methanol	254
CB	*A. melanocarpa*		Poland	x		x		Cold-pressed juice	255
CB	*A. melanocarpa*		Poland	x	x	x		Acidic methanol	249
CB	*A. melanocarpa*		Poland	x	x	x	x	Acidic methanol	256
CB	*A. melanocarpa*	4 biotypes	USA	x	x	x	x	70/30/0.5% acetone/water/acetic acid	251
CB	*A. mitschurinii*	Viking	USA	x	x	x	x	Same as above	251
CB	*A. prunifolia*	4 biotypes	USA	x	x	x	x	Same as above	251
LB	*V. vitis-idae*	cv. Amberland	Canada	x	x	x		80% acidic acetone	252
LB	*V. vitis-idae*	wild	Finland	x	x	x	x	70 % acetone	257
LB	*V. vitis-idae*	5 cultivars	USA	x				100% acetone + 70 % acetone	236
SB	*carpatica*	6 cultivars	Romania		x			70 % acetone	258
SB	*mongolica*	2 cultivars	Estonia		x			Methanol	259
SB	*mongolica*	5 cultivars					x	Acetone/water/acetic acid (80:19.5:0.5, v/v/v)	260
SB	*mongolica*	6 cultivars	Poland and Belarus			x		80 % methanol	246
SB	*rhamnoides**	Wild	Finland				x	Sephadex LH-20 fractioning	261

Berry	*(Sub)species*	*Cultivar/wild*	*Growth location*	*A*	*F*	*PA*	*PAC*	*Extraction*	*Ref.*
SB	*rhamnoides**	cv. Tytti, cv. Terhi	Finland		x			Methanol	259
SB	*rhamnoides**	2 cultivars	Estonia		x			Methanol	259
SB	*rhamnoides**	cv. Hergo			x			Acetone/water (72:25, v/v), Sephadex LH-20 fractioning	244
SB	*rhamnoides**	Wild and 2 cultivars	Finland				x	Acetone/water/acetic acid (80:19.5:0.5, v/v/v)	260
SB	*rhamnoides**		Finland				x	Same as above	262
SB	*sinensis*	Wild	China				x	Same as above	260
SB	*turkestanica*	Wild	India			x		Methanol/water	245

Abbreviations: A, anthocyanins; F., flavonols; PA, phenolic acid; PAC, proanthocyanidins.

* Refers to subspecies *H. rhamnoides* subsp. *rhamnoides*

As in chokeberries, quercetin-3-galactoside was detected as the main flavonol in lingonberry cv. Amberland, with quercetin-3-rhamnoside being present in almost equal amounts. Smaller amounts of quercetin-3-arabinoside was detected (3% of total flavonols) [252]. Likewise, the total flavonol content of lingonberries was similar to chokeberries, 0.26 mg/g FW in Canadian berries [252] and 1.6 mg/g DW in Finnish berries [257].

2.9.3.3 Flavonol content of sea buckthorn

While the profile of flavonol glycosides in sea buckthorn berries varies significantly between different subspecies and varieties [258,260], studies of sea buckthorn berries report high flavonol concentrations, more or less 1 mg/g of the fresh weight (**Table 19**). Flavonol production in berries is increased as a response to abiotic stress, and was increased at increased altitudes and decreased latitudes [263]. The majority of flavonol glycosides in SB berries are isorhamnetin and quercetin derivatives. In addition, myricetin and kaempferol glycosides are present in lower amounts. The typical sugar moieties are rutinose, rhamnose, glucose and sophorose usually as mono-, di- or trisaccharides [103,243,244,247,258]. In addition, acylated flavonols with acyl groups of coumaric acid [244], caffeic acid [244] and sinapic acid [243,258] have been detected.

2.9.3.4 Anthocyanin content of chokeberry and lingonberry

Anthocyanins are responsible for the red, blue, and purple colors in various berries, vegetables, and flowers. These compounds are present typically as glycosides of delphinidin, cyanidin, and pelargonidin. Due to the strong antioxidant capacity both *in vitro* and *in vivo*, intake of anthocyanins has been associated with various benefits to the health [241]

While in sea buckthorn berries the color is due to variation in carotenoid content [10], the color of lingonberries and chokeberries are due to anthocyanins (**Table 20**). Both lingonberries and chokeberries consist mostly of red-colored cyanidin glycosides, cyanidin-3-galactoside being the most abundant in both species, typically followed by cyanidin-3-arabinoside. Apparent differences are due to cyanidin-3-xyloside, which is absent in lingonberries. Additionally, lingonberries show a higher relative amount of cyanidin-3-glucoside (**Table 20**). Moreover, the total anthocyanin content in chokeberries can be 10-fold compared to the content in lingonberries; chokeberries appear dark black while lingonberries have a crimson-red color.

Reported anthocyanin profiles or various cultivars or wild berries of black chokeberries (*A. melanocarpa* or *A. mitchurinii*), despite the genotype and growth location, share surprisingly high similarity, only differing in the total anthocyanin content [253]. This similarity even seems to extend to multiple species of *Aronia* spp [251], suggesting that anthocyanin biosynthesis in *Aronia* spp. is genetically conserved.

Table 16. Total phenolic acid content in *Aronia* spp. cultivars and wild berries and ratio of main phenolic acics. Abbreviations: 3-CQA: 3-caffeyolquinic acid (chlorogenic acid); 5-caffeyolquinic acid (neochlorogenic acid). More information related to references, see **Table 15**.

Species	***Cultivar/wild***	***% of total phenolic acids***		***Total content***	***Ref.***
		3-CQA	5-CQA		
A. melanocarpa	Not reported	65.1	34.9	1.26 mg/ml FW	255
A. melanocarpa	wild	52.1-55.5	44.5-48.0	1.36-1.42 mg/g FW	253
A. melanocarpa	cv. Viking	59.9-60.2	39.8-40.1	1.16-1.67 mg/g FW	253
A. melanocarpa	cv. Nero	57.6-62.1	37.9-42.4	1.08-1.25 mg/g FW	253
A. melanocarpa	cv. Galicianka	56.4-59.5	40.5-43.6	0.74-1.11 mg/g FW	253
A. melanocarpa	Not reported	53.7	46.2	1.21 mg/g FW	249
A. melanocarpa	cv. Galicianka	50.3	1.3	15.3 mg/g DW	250
A. melanocarpa	Not reported	50.9	49.1	5.92 mg/g DW	256
A. melanocarpa	Not reported	66.8	33.2	1.84 mg/100g FW	254
A. arbutifolia	UC021, UC057, UC053	79-100	0-21	5.19-11.20 mg/g DW	251
A. prunifolia	UC047, UC011, UC033, PI578096	56.8-78	22.1-43.0	5.48-17.32 mg/g DW	251
A. melanocarpa	UC007, UC009, PI636375, AMES27010	33.7-70.1	29.9-66.3	7.01-9.86 mg/g DW	251
A. mitschurinii	cv. Viking	60.9	39.2	6.38 mg/g DW	251

Table 17. Phenolic acid composition in berries of various subspecies and cultivars of sea buckthorn (*H. rhamnoides* L). For more details of the sources, see **Table 15**. Abbreviations: GA, gallic acid; PCA, protocatechuic acid; FA, ferulic acid; SA, sinapic acid; HBA, hydroxybenzoic acid; *p*-Cou, *p*-Coumaric acid; CafA, caffeic acid; SalA, salicylic acid; CinA, cinnamic acid.

(Sub)species	***Cultivar/wild***	***Country***	***Main compounds***	***Total content***	***Ref.***
turkestanica	Wild	India	Glycosides (57.3%) > Esters (21.2%) > Free acids (20.0%). GA (66.0%) > PCA (12.7%) > FA (6.5%) > SA (5.1%) > *p*-HBA (3.7%) > *p*-CouA (3.5%)	1.07 mg/g DW	245
not reported	not reported	India	*p*-CouA (35.8-41.9%) > CafA (22.7-34.5%) > FA (20.6-26.9%) > GA (2.8-13.9%)	1.36-1.49 mg/g DW	247
not reported	not reported	Sweden	*p*-CouA (96.1-97.0%) > FA (1.4-2.4%)	5.67-6.54 mg/g DW	247
rhamnoides	Hergo	Germany	PCA (58.3%) > GA (41.7%)	3.6 mg/L FW	248
mongolica	Nadbaltycka	Poland and Belarus	Esters (53.9%) > glycosides (44.2%) > free acids (1.9%). SalA (54.7%) > CinA (17.6%) > p-CouA (9.6%) > GA (5.7%) > FA (4.5%) > PCA (2.9%)	4.57 mg/g DW	246
mongolica	Nevlejena	Poland and Belarus	Esters (55.7%) > glycosides (42.1%) > free acids (2.2%). SalA (69.4%) > GA (11.8%) > CinA (6.3%) > PCA (2.1%) > *p*-CouA (2.0%)	4.75 mg/g DW	246
mongolica	Otradnaja	Poland and Belarus	Esters (60.8%) > glycosides (37.4%) > free acids (1.8%). SalA (54.8%) > GA (27.4%) > PCA (3.5%) > *p*-CouA (5.0%) > FA (2.8%)	3.90 mg/g DW	246
mongolica	Podarok Sadu	Poland and Belarus	Esters (64.5%) > glycosides (34.2%) > free acids (1.3%). SalA (63.2%) > GA (13.9%) > *p*-CouA (10.6%) > 2,5-HBA (2.1%) > CinA (1.9%)	3.87 mg/g DW	246
mongolica	Trofimowskaja	Poland and Belarus	Esters (66.6%) > glycosides (31.8%) > free acids (1.6%). SalA (51.7%) > GA (27.8%) > *p*-CouA (3.5%) > CinA (3.4%) > 2,5-HBA (2.0%)	3.86 mg/g DW	246
mongolica	Hybrid 29-88	Poland and Belarus	Esters (61.7%) > glycosides (36.0%) > free acids (2.3%). SalA (62.8%) > CinA (11.7%) > GA (6.1%) > *p*-CouA (5.0%) > FA (2.8%)	4.07 mg/g DW	246

Table 18. Flavonol glycoside profiles of *Aronia* spp. in various species and cultivars. More details for the references, see **Table 15**. Abbreviations: Q, quercetin; Gal, galactose; Glc, glucose; Rut, rutinose; Rha, rhamnose; Hex, hexose; Nhp, neohesperose.

Species	***Cultivar/wild***	***Main compounds***	***Total content***	***Ref.***
A. melanocarpa	wild	Q-3-Rut (91.7%)	0.24 mg/g FW	253
A. melanocarpa	cv. Viking	Q-3-Rut (87.5%)	0.22 mg/g FW	253
A. melanocarpa	cv. Nero	Q-3-Rut (100%)	0.19 mg/g FW	253
A. melanocarpa	cv. Galicianka	Q-3-Rut (100%)	0.22 mg/g FW	253
A. melanocarpa		Q-3-Gal (40%) > Q-3-Rut (25%) > Q-3-Glc (20%) > Q-3-Vic (10%) > Q-3-Rob (5%)	0.20 mg/g FW	249
A. melanocarpa		Q-3-Gal (36.2%) > Q-3-Glc (21.6%) > Q-3-Rut (15.7%)	1.02 mg/g DW	256
A. melanocarpa	bt. UC007, UC009, PI636375, AMES27010	Q-3-Gal (43.3-53.4%) > Q-3-Glc (30.7-36.6%) > Q-3-Rut (15.3-24.5%)	0.74-1.16 mg/g DW	251
A. prunifolia	UC047, UC011, UC033, PI578096	Q-3-Gal (30.3-45.7%) > Q-3-Rut (23.7-48%) > Q-3-Glc (21.4-34.3%)	0.50-1.37 mg/g DW	251
A. arbutifolia	UC021, UC057, UC053	Q-3-Gal (35.4-41.7%) > Q-3-Rut (34-48.1%) > Q-3-Glc (16.7-26.9%)	0.47-1.19 mg/g DW	251
A. mitschurinii	cv. Viking	Q-3-Gal (45.7%) > Q-3-Glc (38.6%) > Q-3-Rut (15.8%)	1.01 mg/g DW	251
A. melanocarpa	wild	Q-3-Gal (52.6%) > Q-3-Glc (47.5%)	575.5 μg/g FW	252

Table 19. Flavonol glycoside profiles in sea buckthorn berries in various subspecies and cultivars. More details for the references, see **Table 15**.

Subspecies	*Cultivar*	*Main compounds (% of total flavonols)*	*Total content*	*Ref.*
carpatica	Ovidiu	I-3-Hex (27.0%), I-3-(Rha-Glc) (17.9%), I-3-Nhp (16.9%)	10.34 mg/g DW	258
carpatica	Serbanesti	I-3-Nhp (32.5%), I-3-(Rha-Glc) (17.7%), I-3-Hex (12.6%)	9.15 mg/g DW	258
carpatica	Serpenta	I-3-Hex (27.6%), I-3-Nhp (21.8%), I (14.0%)	14.37 mg/g DW	258
carpatica	Sf Gheorghe	I-3-Nhp (20.7%), I-3-(Rha-Glc) (15.1%), I-3-Hex (11.4%)	7.12 mg/g DW	258
carpatica	Tiberiu	I-3-Nhp (20.8%), I-3-Hex (19.9%), I-3-(Rha-Glc) (9.7%)	5.63 mg/g DW	258
carpatica	Victoria	I-3-(Rha-Glc) (16.8%), I-3-Hex (16.8%), I-3-Nhp (13.6%)	8.40 mg/g DW	258
mongolica	Avgustinka	I-3-Glc-7-Rha (22.3%), I-3-Rut (20.3%), I-3-Glc (17.1%)	0.59 mg/g FW	264
mongolica	Trofimovskaya	I-3-Glc-7-Rha (32.4%), I-3-Rut (26.4%), I-3-Glc (16.0%)	0.92 mg/g FW	264
*rhamnoides**	Hergo	I-3-Rut (39.9%), I-3-Glc-7-Rha (24.2%), I-3-Glc (14.3%)	1.20 mg/g FW	264
*rhamnoides**	Leikora	I-3-Glc-7-Rha (32.6%), I-3-Rut (29.6%), I-3-Glc (15.2%)	1.69 mg/g FW	264
*rhamnoides**	Terhi	I-3-Glc-7-Rha (38.0%), I-3-Rut (33.0%), I-3-Glc (9.6%)	0.94 mg/g FW	264
*rhamnoides**	Tytti	I-3-Glc-7-Rha (35.2%), I-3-Rut (20.0%), I-3-Glc (11.8%)	0.99 mg/g FW	264

Abbreviations: I, isorhamnetin; Glc, glucose; Rut, rutinose; Rha, rhamnose; Hex, hexose; Nhp, neohesperose.

* Refers to subspecies *H. rhamnoides* subsp. *rhamnoides*

Table 20. Anthocyanin profile and total anthocyanin content in chokeberries and lingonberries of different origins.

		% of total anthocyanin content					
(Sub)species	***Cultivar/wild***	***Cy-3-Gal***	***Cy-3-Ara***	***Cy-3-Xyl***	***Cy-3-Glc***	***Total content***	***Ref.***
A. melanocarpa		64.6	29.4	3.8	2.1	6.08 mg/ml FW	[255]
A. melanocarpa	wild	68.0-68.9	25.8-26.0	2.9-3.5	2.4	2.48-3.52 mg/g FW	[253]
A. melanocarpa	cv. Viking	66.9-68.9	25.4-27.2	3.3-3.6	2.0-2.6	4.19-4.39 mg/g FW	[253]
A. melanocarpa	cv. Nero	66.0-67.7	27.2-27.9	3.0-4.2	1.8-2.2	2.72-5.49 mg/g FW	[253]
A. melanocarpa	cv. Galicjanka	68.0-70.3	24.5-26.1	3.0-3.4	2.3-2.5	1.50-3.22 mg/g FW	[253]
A. melanocarpa		71.8	22.0	4.9	1.3	5.28 mg/g FW	[249]
A. melanocarpa		65.5	29.7	2.7	2.2	19.59 mg/g DW	[256]
A. melanocarpa	cv. Galicjanka	68.1	27.4	2.4	1.9	121.6 mg/g DW	[250]
A. melanocarpa		65.5	30.4	2.1	2.1	4.81 mg/g FW	[254]
A. melanocarpa	UC007, UC009, PI636375, AMES27010	63.4-97.6	1.3-32.8	0-2.5	1.3-1.6	3.37-14.87 mg/g DW	[251]
A. prunifolia	UC047, UC011, UC033, PI578096	65.3-97.3	1.6-34.2	0-1.8	1.3-1.5	2.40-3.99 mg/g DW	[251]
A. arbutifolia	UC021, UC057, UC053	85.8-96.7	3.4-12.6	0	0-1.7	0.48-0.82 mg/g DW	[251]
A. mitschurinii	cv. Viking	64.7	29.2	2.8	3.4	13.92 mg/g DW	[251]
A. melanocarpa	wild	39.7	45.0	14.8	0.5	3166.5 µg/g FW	[252]
V. vitis-idae	cv. Ida	76	14	0	10	0.52 mg/g FW	[236]
V. vitis-idae	cv. Koralle	68	14	0	19	0.28 mg/g FW	[236]
V. vitis-idae	cv. Linnea	83	10	0	7	0.27 mg/g FW	[236]
V. vitis-idae	cv. Sanna	82	9	0	9	0.43 mg/g FW	[236]
V. vitis-idae	cv. Sussi	85	8	0	7	0.42 mg/g FW	[236]
V. vitis-idae	cv. Amberland	80	10	0	2.4	0.61 mg/g FW	[252]

2.9.3.5 Complex polyphenols

Complex, polymeric polyphenols are divided into two subclasses, hydrolysable and condensed tannins. The former group refers to ellagitannins and gallotannins, which are built up from ellagic acid and gallic acid monomers, respectively, bound with ester bonds, and usually have a carbohydrate molecule at the center of the compound [265]. As discussed earlier, *L. plantarum* produces enzymes that can hydrolyze the ester bonds in gallotannins (**Section 2.4.4**). However, in sea buckthorn, lingonberry and chokeberry, the main oligomeric and polymeric polyphenols are condensed tannins, and to be more specific, proanthocyanidins, which consists of two or more subunits of flavan-3-ol, catechin, epicatechin, or epigallocatechin.

In lingonberries, flavan-3-ols and proanthocyanidins (PAC) are by far the most abundant phenolic group. Different flavan-3-ol fractions of lingonberry extract showed that A-type dimers were the most common oligomeric PAC, while in the polymeric fraction the mean degree of polymerization was 32 [257]. In *Aronia* spp., PAC are among the most abundant phenolic compounds, especially in the species that produce purple and red berries i.e. produce less anthocyanins compared to the black chokeberry. However, even in black chokeberry, PAC are present at equal amounts to anthocyanins [251]. The PAC in *Aronia* spp. are present almost entirely as polymeric (degree of polymerization (DP) > 10), ranging between 96.9-99.9 % of the total flavan-3-ol content [250,251,256].

Sea buckthorn berries, while showing a lower content of PAC compared to lingonberries and chokeberries, still contain substantial amounts of flavan-3-ols (**Table 21**). Structure and concentrations of oligomeric PAC (DP < 5) have mainly been reported in sea buckthorn. Berries of ssp. *rhamnoides* typically report the highest ratio of PAC trimers [260,262], and the epigallocatechin trimer was the most abundant in Finnish berries belonging to this subspecies [261]. In addition to oligomeric flavan-3-ols, the presence of substantial amounts of polymeric PAC in sea buckthorn berries have been noted [261]. However, no research discovered regards the content or structural analysis of polymeric PAC in sea buckthorn berries.

Table 21. Polymeric flavan-3-ol (proanthocyanidin, PA) content in sea buckthorn (SB), chokeberries (CB) and lingonberries (LB) at various growth locations. More information related to the references, see **Table 15**.

Berry	***Subspecies***	***Cultivar/wild***	***Type of PA***	***Main compounds***	***Concentration***	***Ref.***
CB	*A. arbutifolia*	Three biotypes		Polymers (DP > 10) 99.6 % of the total	1.10-3.67 mg/g DW **, 12.15 mg/g DW ***	251
CB	*A. melanocarpa*	cv. Galicjanka		Polymeric (96.9%) > epicatechin (1.7%) > catechin (1.2%)	103 mg/g DW *	250
CB	*A. melanocarpa*			Mean degree of polymerization 23	51.82 mg/g DW **	256
CB	*A. melanocarpa*	Four biotypes	All B-type	Polymers (DP > 10) 99.9 % of the total	1.56-2.33 mg/g DW **, 10.34 mg/g DW ***	251
CB	*A. mitschurinii*	cv. Viking	All B-type	Polymers (DP > 10) 99.9 % of the total	3.26 mg/g DW **, 10.34 mg/g DW ***	251
CB	*A. prunifolia*	Four biotypes	All B-type	Polymers (DP > 10) 99.6 % of the total	1.72-2.02 mg/g DW **, 9.28 mg/g DW ***	251
LB	*V. vitis-idae*	Wild	All A-type	Catechin main flavan-3-ol. Fraction 1: A-type dimer 20.3%. Fraction 10: polymeric fraction with mDP of 32.	12.30 mg/g DW **	257
SB	*mongolica*	cv. Chuiskaya	All B-type	Dimer (39.0%) > Trimer (36.4%) > Tetramer (24.6%)	5.70 mg/g DW ***	260
SB	*mongolica*	cv. Oranzhevaya	All B-type	Trimer (37.4%) > Dimer (33.6%) > Tetramer (28.9%)	7.00 mg/g DW ***	260
SB	*mongolica*	cv. Vitaminaya	All B-type	Dimer (38.7%) > Trimer (37.0%) > Tetramer (24.3%)	6.81 mg/g DW ***	260
SB	*mongolica*	cv. Prevoshodnaya	All B-type	Trimer (38.6%) > Dimer (34.8%) > Tetramer (26.6%)	8.80 mg/g DW ***	260
SB	*mongolica*	cv. Prozcharachnay	All B-type	Dimer (42.2%) > Trimer (35.9%) > Tetramer (21.9%)	3.89 mg/g DW ***	260
SB	*rhamnoides*	Wild	All B-type	EGC trimer > EC dimer > EC-EGC dimer > EC-EGC-EC trimer > EGC tetramer	30 µg/g FW **	261

Berry	***Subspecies***	***Cultivar/wild***	***Type of PA***	***Main compounds***	***Concentration***	***Ref.***
SB	*rhamnoides*	Wild	All B-type	Trimer (38.2-44.6%) > Tetramer (27.0-33.3%) > Dimer (22.1-34.9%).	8.94-16.43 mg/g DW ***	260
SB	*rhamnoides*	cv. Tytti	All B-type	Trimer (37.2-39.5%) > Tetramer (31.0-33.8%) > Dimer (26.8-31.7%)	6.42-19.41 mg/g DW ***	260
SB	*rhamnoides*	cv. Terhi	All B-type	Trimer (42.2-43.2%) > Tetramer (30.3-36.0%) > Dimer (20.8-27.3%)	5.94-15.15 mg/g DW ***	260
SB	*rhamnoides*	cv. Terhi	All B-type	Trimer (43.9-44.1%) > Tetramer (25.4-32.9%) > Dimer (23.2-30.5%)	3.69-9.37 mg/g DW ***	262
SB	*rhamnoides*	cv. Tytti	All B-type	Trimer (40.5-43.8%) > Tetramer (27.1-30.4%) > Dimer (29.1-29.2%)	4.28-9.67 mg/g DW ***	262
SB	*rhamnoides*	Wild	All B-type	Trimer (41.1-43.1%) > Dimer (31.0-32.1%) > Tetramer (25.9-26.8%)	4.53-7.73 mg/g DW ***	262
SB	*sinensis*	Wild	All B-type	Trimer (35.7-42.1%) > Dimer (25.8-36.8%) > Tetramer (23.6-33.3%)	5.74-11.73 mg/g DW ***	260

* Quantified with phloroglucinolysis method

** Quantified with HPLC

*** Quantified with colometric method (*p*-Dimethylaminocinnamaldehyde)

2.9.4 Volatile compound profile of sea buckthorn berries

Depending on the subspecies and growth site, the five most abundant VOCs in sea buckthorn berries are esters 3-methylbutyl 3-methylbutanoate (3-MB-3MB) and ethyl esters of 3-methylbutanoic acid (E-3MB), 2-methylbutanoic acid (E-2MB), hexanoic acid (E-HA), and octanoic acid (E-OA) (**Fig. 18**) [266–268]. Leung and Marriott (2015) [266] reported high content of 3-methylbutyl benzoate in berries grown in the UK while only trace amounts were detected in berries studied by Socaci et al. [267] and Tiitinen et al. [268] (< 1 % and <2.4 % of the total VOC, respectively). Additionally, Tiitinen et al. 2006 reported relatively high amounts (up to 10% of the total VOC content) of 3-methylbutyl hexanoate in Russian sea buckthorn cultivars grown in Finland [268]. All the previously mentioned compounds have a fruity odor descriptor [52].

When 12 cultivars and wild biotypes of sea buckthorn (subspecies *carpatica*) grown in Romania where compared, it was observed that the content of E-2MB and E-HA had inverse correlation with the content of both E-3MB and 3-MB-3MB. In addition, a weaker but yet negative correlation was also detected between E-3MB and 3-MB-3MB, suggesting competing pathways in the biosynthesis of these volatile esters [267].

Significant year-to-year variations in VOC profiles of sea buckthorn berries have been observed (**Fig. 18**) [266,268], highlighting that VOC formation in sea buckthorn berries is substantially impacted by environmental factors. A positive correlation between the total VOC content and number of sunshine hours during the growth season was observed, suggesting that increased radiation is related to volatile formation in sea buckthorn [266]. Similar volatile profiles were reported by [266–268].

2.9.5 Relationship between chemical composition and sensory value

Multivariate modeling has been used to associate sensory and chemical parameters of different *H. rhamnoides* subspecies and hybrids and found that the sugar/acid ratio (0.39-1.04) predicted the pleasantness of sea buckthorn better than sugar content alone [269]. A sugar derivative, ethyl β-d-glucopyranoside (E-β-G), has been detected in sea buckthorn berries. Variation between 0.6 to 19.8 g/L FW in sea buckthorn berries was reported while the pure compound had a taste threshold of 1.1 ± 1.3 g/L in water with a bitter taste [264]. Moreover, the bitterness of the juice was correlated with E-β-G content as well as with the ratios between the E-β-G/acids and E-β-G/sugars [264].

An addition of 5 % sucrose decreased sourness and improved the preference for chokeberry juice from weak dislike to weak like. However, neither sweetening with sucrose nor flavoring with ethyl butyrate blocked the juice astringency. Moreover, sourness, astringency and bitterness were associated with

flavonols and hydroxycinnamic acids. Interestingly, proanthocyanidins correlated with the hedonics and sweetness [13].

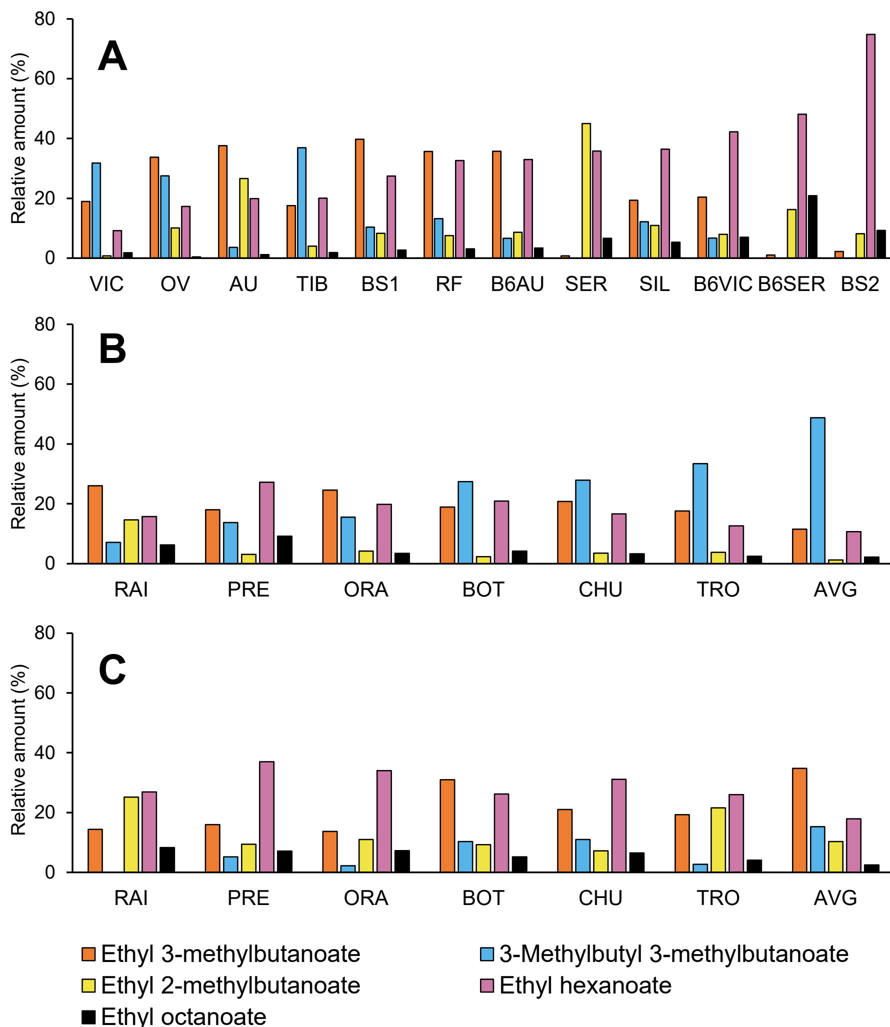


Fig. 18. Profiles of selected VOC (based on abundance) between cultivars and biotypes of sea buckthorn belonging to A) the subspecies *carpatica* grown in Romania [267], and in berries of the subspecies *mongolica* (RAI, *rhamnoides*) collected in Finland during the years B) 2002 and C) 2003 [268]. The Y-axis represents the relative amounts in relation to the total VOC content. For full cultivar names and additional details, see **Supplementary Table S2**.

3 AIMS OF THE STUDY

The overall goal of this work was to develop a protocol for effective fermentation with *L. plantarum* for berry materials with low consumer value. The goals were to decrease acidity with malolactic fermentation, modify the phenolic compound composition, affect the volatile profile in a positive manner, and to generate compounds with functional (e.g., antimicrobial, or bioactive) properties.

The goal was to screen for whether or not fresh or enzymatically treated juices of sea buckthorn, lingonberry, or chokeberry are suitable raw materials for fermentation with *L. plantarum*. The metabolism of sugars, organic acid, sugar alcohols, and phenolic compounds were determined with qualitative and quantative analysese (**I**).

Changes in the volatile compound composition of sea buckthorn juice was determined to screen for potential positive or negative changes in the aroma profile during fermentation. The importance of strain selection, starter pH, fermentation time, and basal medium composition in relation to the volatile profile were discussed (**II**).

The study endeavored to evaluate the suitability of NMR-based metabolomics as a holistic analytical tool for fermented sea buckthorn materials. Change in the chemical composition of sea buckthorn during fermentation was compared to the existing knowledge related to metabolic systems of *L. plantarum*. The importance of strain selection, starter pH, fermentation time, and basal medium composition were discussed in relation to the metabolic profiles of fermented juices (**II**).

4 MATERIALS AND METHODS

4.1 Berry materials

In this dissertation, the focus was on the berries of sea buckthorn, chokeberry, and lingonberry, all of which have been reported to contain substantial amounts of health-promoting bioactive compounds [10–12] while having a low consumer value [13–15]. Whilst sea buckthorn [228] and chokeberries [226] have a high malic acid content, the most abundant acid in lingonberries is citric acid [229]. Lingonberry is not therefore optimal for the MLF, but it is by far the most valuable non-timber forest resource in Finland [270]. Due to the high commercial value, lingonberry is a relevant target for product development and was therefore included in this work.

In **Study I**, frozen chokeberries (*Aronia mitschurinii* 'Viking') were purchased from Vinkkilän luomutuote Oy (Vehmaa, Finland). Frozen wild lingonberries (*Vaccinium vitis-idaea*) were purchased from RH Foods OÜ (Tallinn, Estonia). Origin of the berries was Lithuania. Frozen sea buckthorn berries (*Hippophaë rhamnoides* subspecies *mongolica*) originating from Southern Estonia were purchased from Astelpajutooted OÜ (Tõrva, Estonia). In **Study II** and **III**, frozen sea buckthorn berries (subspecies *mongolica*), mixture of cultivars 'Ljubitelskaja' and 'Prozrachnaya', were acquired from a professional farmer (Vinkkilän luomutuote, Vehmaa, Finland). In all cases, the berries were frozen right after picking and stored at –20 °C until use.

4.2 Juice preparation

In **Study I**, the frozen berries (700–1050 g per batch) were thawed in a microwave at 650W for 5 min. The berries were made into a mash with an immersion blender. Juice was extracted from the berry mash with a central screw basket press. Juices were pasteurized immediately after the extraction, stored overnight at +4 °C, and inoculated the next day.

Frozen sea buckthorn berries were thawed in a microwave at 600 W for 3.5 min. Next, the berries were made into a mash with a Bamix immersion blender. The juice was extracted from the mash with a fruit press (Chef Titanium XL with AT644 attachment, Kenwood, UK) in batches of ~400 g of mash, and the juice was filtered through a cheesecloth to remove solids. Thereafter juices were pooled, divided into aliquots for each fermentation batch, and stored at –20 °C until use (**II**, **III**).

4.2.1 Pasteurization

After extraction with pressing, juices were pasteurized in an autoclave (Systec D-150, Linden, Germany) at 85 °C for 5 min. After the pasteurization, the juices were immediately cooled down in an ice bath (**I**). In **Study II** and **III**, prior to pasteurization, the juices were diluted 1:1 (w/w) and divided into 30 mL aliquots in individual glass vials. The juice samples were pasteurized in a water bath (temperature ~96 °C) until temperature of the juices reached 90 °C, and this was followed by cooling the juices in an ice bath until 10 °C. The juice temperature was monitored with a thermometer (TM-947SD, Lutron Electronics, South Korea) coupled with a thermocouple probe.

4.2.2 Other treatments

In **Study I**, two types of juices were prepared for each berry: a fresh juice and an enzyme-treated juice. The latter was prepared by adding 200 µL/kg of pectinase (Pectinex® Ultra SPL, Novozyme, Bagsvaerd, Denmark) to the berry mash, and the mash was subsequently incubated at 45 °C for 4 h, followed by extraction with a manual juice press. In the next two studies, two types of juice were used for fermentation, one with natural pH (2.7) and the other with pH adjusted to 3.5 with 1 M NaOH (**II**, **III**).

4.3 Bacterial strains

Initially, *Lactiplantibacillus plantarum* strains DSM 20174^T, DSM 10492, DSM 100813 as well as *Lactiplantibacillus argentoratensis* strain DSM 16365^T were used in the fermentations. In addition, dehydrated cells of *Oenococcus oeni* strains LAB6, LAA1, and B2013, were provided kindly by Lallemand Inc. (Montreal, Canada) (**I**).

In the later studies, no *O. oeni* strains were used in any of the fermentations but additional *L. plantarum* strains were purchased (DSM 1055 and DSM 13273) (**II**, **III**). All *L. plantarum* stocks were acquired as freeze-dried cultures from the DSMZ (Braunschweig, Germany), and were revived according to the manufacturer's protocol. The revived cultures were stored as 10% (v/v) glycerol stocks at –80 °C until use (**I**, **II**, **III**). The dehydrated *O. oeni* cells were revived in sterile saline for 30 min. at RT prior to inoculation (**I**).

4.4 Fermentation

The *L. plantarum* starter culture for the fermentation was prepared by inoculation of 250 mL of general edible medium (GEM) (soy peptone 30 g L^{-1}, dextrose 20 g L^{-1}, yeast extract 7 g L^{-1}, $MgSO_4 \times 7\ H_2O$ 1 g L^{-1}, in potassium phosphate

buffer 0.01 M, pH 6.3 ± 0.2) with a scrape from a glycerol stock, followed by incubation at +30 °C for 24 h. The cells were collected with centrifugation (3,360 × g, 5 min, 4 °C) and washed twice with sterile saline. Finally, 1 mL of concentrated cell suspension was added to a 100 mL juice sample, leading to to initial cell count of approximately 1×10^8 CFU/mL. In addition, in **Study I**, dehydrated *O. oeni* cells (0.5 g/10 mL) were revived in sterile saline with gentle mixing for 30 min. at RT, and 200 µL of cell suspension was added to a 100 mL juice sample, with expected initial cell count of 10^7 CFU/mL. All samples were fermented for 72 h at +30 °C in iCinac equipment (Unity Scientific, Milford, USA) equipped with InLab® Smart Pro-ISM probes for iCinac (ph/redox/Temp) (Mettler Toledo) and TW8 water bath (Julabo, Seelbach, Germany). All fermentations were prepared in duplicates (**I**).

Study II and **III**, MRS plates were initially inoculated with a scrape from glycerol stock and incubated for 36 h at +30 °C. Next, the growth media, either general edible medium (GEM) (dextrose 30 g L^{-1}, soy peptone 20 g L^{-1}, yeast extract 7 g L^{-1}, $MgSO_4 \times 7\ H_2O$ 1 g L^{-1}, $MnSO_4 \times H_2O$ 0.05 g L^{-1} in potassium phosphate buffer 0.01 M, pH 6.3 ± 0.2) or cell acclimation medium (GEM with additional 4 g/L of L-malic acid, pH adjusted to 4.5 ± 0.1) was inoculated with a single culture from the MRS plate. Inoculated medium was incubated for 24 h at +30 °C. Next, the cells were collected *via* centrifugation (4,500 × g, 5 min., RT) and washed twice with PBS (pH 7.4). The cell count for the fermentation was standardized with optical density at 600 nm. The target initial cell count in a juice sample was 2×10^8 CFU/mL. The juice samples were fermented at +30 °C for 36 or 72 hours in a Memmert IF-110Plus incubator. All fermentations were prepared as triplicates.

Prior to fermentation, the viable cell count of the starter cultures was confirmed with a viable colony count. Colony counts between 30–300 on each plate were considered acceptable for enumeration.

4.5 Sample preparation

Simple carbohydrates and non-volatile organic acids were analyzed as TMS-derivatives. Briefly, aliquots of 300 µL of 5% berry juice (v/v in RO-water) with xylitol (0.6572 M) and tartaric acid (0.66628 M) were dried under nitrogen flow, followed by an overnight desiccation to remove residual water. Dry samples were derivatized with chlorotrimethylsilane reagent with pyridine and hexamethylsilazane (Tri-Sil HTP, Thermo Scientific, Bellefonte, PA, USA) (**I**).

To extract the phenolic acids and flavonols, approximately 11 g of juice sample was extracted with 10 mL of ethyl acetate four times. The sea buckthorn juice and ethyl acetate formed an emulsion, and 1–2 mL of 4M NaCl was used to separate the two phases. The extracts were evaporated until dryness in a rotary

evaporator (Hei-VAP, Heidolph GmbH, Schwabach, Germany) (35 °C, 100 rpm) and re-dissolved to 3 mL of methanol. The extraction was performed in quadruplicates. Samples were filtered (0.45 µm) and stored at –80 °C until analyses. For analysis of anthocyanins, juice samples were filtered (0.2 µm) and diluted if needed prior to analysis (**I**).

For headspace volatile analysis, 2 mL of juice sample was spiked with 10% (w/v) NaCl and 10 µL ISTD (ethyl propionate 100 ppm; nonane 200 ppm) and subsequently analyzed with the GC-MS instrument (**II**).

For the NMR analysis, a clear aqueous phase from turbid sea buckthorn juice was collected by removing majority of the solids via centrifugation (14,000 × g, 3 min, +4 °C), followed by centrifugal filtration to remove the residual solids. Next, 300 µL of clarified juice, 70 µL of Chenomx IS-2 (5 mM DSS-d6, 0.1% (w/v) NaN_3 in D_2O, pH 7.0), 70 µL of 1.5M K_2HPO_4/KH_2PO_4 buffer (pH 6.5), 70 µL of 1 M maleic acid standard were mixed, pH adjusted to 6.00±0.02 and brought to final volume of 700 µL with dH_2O. Finally, 650 µL of the sample was transferred to a 5 mm NMR tube (**III**).

4.6 Analytical methods

Carbohydrates and organic acids were analyzed with a GC-FID instrument. The GC equipment consisted of an autosampler (AOC-20 s) with an autoinjector (AOC-20i), a column oven (Shimadzu GC-2010Plus) and a flame ionization detector (Shimadzu, Kyoto, Japan). All samples were prepared in triplicates. TMS-derivatives of sugars and organic acids were separated with a nonpolar capillary column SPBTM-1 (30m×0.25mm ID, liquid film 0.25 µm, Supelco, Bellefonte, PA, USA). The analysis was carried out in a split mode with a split ratio of 15:1, and the injection volume 1 µL (**I**). In addition, the concentrations of L-malate, L-lactate, and D-lactate of sea buckthorn juice before and after fermentation were determined using K-LMAL, K-LATE, K-DATE enzyme kits (Megazyme, Bray, Ireland), respectively (**II**).

Flavonols, phenolic acids and anthocyanins were analyzed with a HPLC-DAD instrument. The HPLC-DAD instrument consisted of a Shimadzu (Shimadzu Corporation, Kyoto, Japan) SIL-20AC auto sampler, DGU-20A degasser unit, a sample cooler, two LC-20AD pumps, a CTO-20AC column oven, an SPD-M20A diode array detector, and a CBM-20A central unit. The system was operated using the LabSolutions Workstation software. Analytes were separated with a XB Aeris Peptide C18 column (3.60 µm, 150mm×4.60 mm, 100 Å; Phenomenex Inc, Torrance, CA) combined with a Phenomenex Security Guard Cartridge Kit (Torrance, CA). A binary gradient mobile phase system was used. Solvent A was a mixture of water:formic acid (99:0.1, v/v), and solvent B was acetonitrile:formic acid (99:0.1, v/v). The flow rate of the mobile phase was

1 mL/min, the column temperature 30 °C, and the injection volume 10 μL. The solvent gradient program was: 0–15 min, 2–18% B; 15–25 min, 18% B; 25–40 min, 18–60% B; 40–45 min, 60–2% B; 45–50 min, 2% B. UV–vis absorption spectra were measured within the wavelength range of 190–600 nm with the DAD (**I**).

Juice samples were filtered (0.2 μm), diluted if needed, and subsequently injected directly to the HPLC system. The column was a reverse-phase Kinetex C18 (2.60 μm, 100mm×3.60 mm, 100 Å, Phenomenex Inc, Torrance, CA) combined with Phenomenex Security Guard Cartridge Kit (Torrance, CA). A binary gradient mobile phase system was used. Solvent A was 5% formic acid in MilliQ, water, while solvent B was 5% formic acid in acetonitrile. The flow rate of the mobile phase was 1 mL/min, the column temperature 30 °C, and the injection volume 10 μL. The solvent gradient program was: 0–10 min, 5–8% B; 10–15 min, 8% B; 15–20 min, 8–9% B; 20–22 min, 9–12% B; 22–35 min, 12–60% B; 35–40 min, 60–5% B; 40–45 min, 5% B. Photo absorption spectra were recorded at the wavelength of 515 nm with the DAD (**I**).

Headspace volatiles were collected with solid phase microextraction (SPME) with a 2 cm DVB/CAR/PDMS fiber (50/30 μm, Supelco, Bellefonte, PA) at 45 °C for 20 min. Prior to the headspace volatile collection, the juice sample was incubated 10 min at 45 °C and the fiber conditioned at 230 °C. Analytical instrument of the headspace volatiles consisted of a Trace 1310 gas chromatograph coupled with a TSQ 7000 single quadropole mass spectrometer (Thermo Fisher Scientific, Waltham, MA). The gas chromatograph instrument was equipped with either DB-WAX polar capillary column (60m×0.25mm i.d.×0.25 μm film thickness, J&W Scientific, Folsom, CA) or SPB-624 mid-polarity capillary column (60m×0.25mm i.d.×1.4 μm film thickness, Supelco, Bellefonte, PA). Mass spectra were detected in electron impact mode at 70 eV with a full scan mode (scan range of 33–300 m/z) and a scan speed 0.2 s. The temperatures of the MS transfer line were 200 °C and 210 °C for DB-WAX and SPB-624 columns, respectively (**II**).

In **Study III**, spectra were recorded using a 600 MHz AVANCE-III NMR-system (Bruker Biospin, Rheinstetten, Germany) equipped with a CryoProbe Prodigy TCI (Bruker Biospin) and an automated sample changer SampleJet. Instrument was operated using Topspin (version 4.1.0) and IconNMR softwares (Bruker Biospin). The proton spectra were acquired at 298.2 K with 1D NOESY pulse program with presaturation (*noesygppr1d*). The following parameters were used: size of the FID, 64k; spectral width, 14 ppm; number of scans, 128; number of dummy scans, 4; 90° proton pulse length 10.98 μs; relaxation delay, 5 s; mixing time, 0.10 s. Multiplicity edited ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC) using echo/antiecho detection and gradient pulses (*hsqcedetgpsisp2.3*) was acquired with the following parameters: 90° pulse

values, 8 µs (proton) and 15 ls (carbon); relaxation delay, 2 s; spectral width, 165 ppm (f1) and 16 ppm (f2); data points, 256 increments of 2 k; number of scans, 32. ^{1}H–^{13}C heteronuclear multiple-bond connectivity (HMBC) with absolute value detection (*hmbcgplpndqf*) was acquired with the parameters: spectral width, 220 ppm (f1) and 10 ppm (f2); data points, 128 increments of 2 k; number of scans, 64. Homonuclear ^{1}H–^{1}H COSY (*cosygpppqf*) was acquired with 2048 data points with increments of 128 and with 16 scans.

4.7 Identification

Authentic standards in addition to literature references were utilized for the identification various sugars and acids from both the fresh and the fermented berry juices (**I**). From the same juices, the flavonol glycosides, anthocyanins, and hydroxycinnamic and hydroxybenzoic acids were identified using UV–*vis* spectra, MS and MS/MS spectra, retention times and by comparison to authentic standards and literature references (**I**).

From both the fresh and the fermented sea buckthorn juices, the volatile compounds were identified by comparing the mass spectra with standard NIST 08 library, literature data and Kovats retention indices (RI). The RIs of the volatile compounds were calculated based on the retention times of C5–C30 alkane mixture (Sigma-Aldrich, St. Louis, MO) determined using the same gas chromatographic conditions (**II**).

From ^{1}H-NOESY NMR spectra of the fresh and fermented sea buckthorn juices, the metabolite annotation was based on the chemical shift, *J*-coupling, heteronuclear coupling (HSQC, HMBC), and homonuclear coupling (^{1}H–^{1}H COSY). Spectrum databases of Chenomx NMR Suite software, the Human Metabolomics Database (http://www.hmdb.ca/) and the Biological Magnetic Resonance Data Bank (http://www.bmrb.wisc.edu/) were used as the main references in addition to other literature sources (**III**).

4.8 Quantification

In the **Study I**, quantification in GC-FID analyses was made with the internal standards, xylitol for the sugar compounds, and tartaric acid for the organic acids. In addition, correction factor for each compound was determined with external standards with known concentrations. Quantification of the phenolic compounds in **Study I** was performed by comparing the peak areas at a selected wavelength to the linear calibration curves of the external standards. Quercetin derivatives were quantified with a calibration curve constructed with quercetin 3-*O*-rhamnoside (detection wavelength 360 nm, concentration range for constructing calibration curve: 23.7–3791.4 µM, R^2, 0.999), isorhamnetin derivatives with

isorhamnetin 3-*O*-rutinoside (360 nm, 12.2–4867.5 µM, R^2, 0.9993), hydroxycinnamic acids with chlorogenic acid (320 nm, 81.6–13062.0 µM, R^2, 0.9995) and hydroxybenzoic acids with protocatechuic acid (260 nm, 180.1–28808 µM, R^2, 0.9995). Anthocyanins were quantified similarly as described above, using cyanidin-3-*O*-glucoside as an external standard. A calibration curve (R^2 0.9997) was constructed by analysis of standard solutions of a concentration range of 0.46–1155.34 µM at wavelength of 515 nm.

From the SPME-GC-MS results, the individual volatile compounds were semi-quantified (µg/L) by comparing the area of the base peak ion to the area of the base peak ion of ethyl propionate (internal standard), which was selected due to the low sample to sample variation in peak area and due to the high number of esters present in the sea buckthorn juice (**II**).

Quantification of the selected metabolites from the 1D-NOESY spectra was performed using the Chenomx software and its profiling tool. The results were normalized using the peak area of the internal standard, maleic acid (1 mM) (**III**).

4.9 Statistical analysis

Results were reported as mean ± standard deviation, determined from biological duplicates in the **Study I** and from biological triplicates in the **Studies II** and **III**. The Tukey's test for population with equal variances was performed for a multiple comparison. The differences reaching confidence level of $p < 0.05$ was considered as statistically significant. Statistical analyses were performed initially with software R (The R Foundation for Statistical Computing, Vienna, Austria) using the library *agricolae* with R version 3.2.3 [271] (**I**, **II**), and later with the library *multcompView* with the R version 4.1.0 (**III**). Default parameters of the package was used. The following statistical analyses were carried out using IBM SPSS 25.0 (SPSS, Chicago, IL, USA): A Student's *t*-test was used to compare the juice yields, contents of the lactic and L-malic acid and rate of malolactic conversion between enzyme treated and fresh juices. General linear models were used to compare the differences in malolactic conversion rates dependent on the bacterial strain and juice type (**I**).

To study differences between the *L. plantarum* strains (X = 6, n = 24) and the impact of fermentation time (0 h, n = 12; 36 h, n = 78; 72 h, n = 78), juice pH, and growth media as combined variable (X = 4, n = 36) in relation to the sums of volatile compound subgroups, IBM SPSS 26.0 (SPSS, Chicago, IL, USA) was used. In addition, principal component analysis (PCA) was carried out using the software Unscrambler X (version 11, Camo Inc., Norway). This was used to illustrate the relationship between the volatile composition and the treatments applied in production of the fermented sea buckthorn juice (**II**).

All ^{1}H-NMR spectra were processed and analyzed using the Chenomx NMR Suite Professional software version 7.0 (Chenomx Inc. Edmonton, AB, Canada). DSS was used as an internal standard for chemical shift referencing (set to 0 ppm). The manual phase and baseline correction were performed on each of the spectra. All the ^{1}H-NMR FIDs were zero-filled to 64k data points and line broadned 0.3 Hz. After processing, the spectra were divided into 0.02 ppm-sized bins, the water region removed, and the data normalized to the total spectral area. To align the spectral data for untargeted statistical analyses, the dataset was imported into MATLAB software (version 2020B, Mathworks Inc., Natick, MA, USA) and processed using the icoshift algorithm. Principal component analysis and orthogonal principal least squares discrimination (OPLS-DA) of processed and aligned spectra were carried out using SIMCA (version 16, Umetrics, Umeå, Sweden). Pareto scaling and mean centering were applied to the datasets. The validation of the OPLS-DA models was performed with internal validation of 100 permutations as well as by determining explained variation (R^2Y_{cum}) and predictive ability (Q^2Y_{cum}) (**III**).

Besides the multivariate methods, in **Study III**, paired *t*-test was used to compare the means of individual metabolites, grouped by growth medium with fermentation time and the starter pH set as constant (72 h and 3.5, respectively). To compare the metabolic responses based on the starter pH of sea buckthorn juice, hierarchical clustering heatmap analysis was performed with MetaboAnalyst 5.0 open source platform (https://www.metaboanalyst.ca/) [272]. Data was normalized with auto-scaling.

5 RESULTS AND DISCUSSION

5.1 Sugar, sugar alcohols, and organic acid content of the berry materials

The pH, sugar, sugar alcohol, and organic acid content was determined in the juices made from sea buckthorn, chokeberries, and lingonberries. The lowest pH was observed in lingonberry 2.68, followed by sea buckthorn (2.87) and chokeberry (3.39). Despite this, the highest total acid content (w/v) was measured from the SBJ (18.2 g/L). Chokeberries had the lowest acid content (10.0 g/L) after lingonberry juice (15.9 g/L). In accordance with earlier reports (**Table 14**), the main acids in both SBJ and chokeberry juices were L-malic acid and quinic acid, while the main acid in lingonberry juice was citric acid.

Highest sugar content (sugars + sugar alcohols) was measured from the chokeberry juice (56.4 g/L), followed by lingonberry (32.8 g/L) and sea buckthorn (15.5 g/L). The main sugars in chokeberries were sorbitol and glucose, while glucose and fructose were the most abundant in both sea buckthorn and lingonberries. Pectinolytic enzyme treatment prior to the juice extraction significantly increased both the total sugar and the total acid content of lingonberry juice (**I**).

The sugars and acids of SBJ were analyzed also in a later study (**III**). In the first study, Estonian berries were used (**I**) while in the later study, Finnish sea buckthorn berries were used (**III**). In both instances, the berries belonged to the subspecies *mongolica* but were mixture of several cultivars of a Russian origin. The juice made from Finnish berries had lower pH and lower contents of glucose, fructose, ascorbic acid, and L-malic acid compared to the juice made from the Estonian berries. Only the quinic acid content was reported to be higher in the Finnish berries (**III**) (**Table 22**).

Table 22. Concentrations of the sugars, sugar alcohols, and organic acids in fresh sea buckthorn juice (**I**, **III**). Juices were diluted with water 1:1 in both instances.

Compound / value	*Study I, g/L*	*Study III, g/L*
pH	2.87 ± 0.01	2.70 ± 0.01
Glucose	11.27 ± 0.13	4.75 ± 0.04
Fructose	2.11 ± 0.06	1.20 ± 0.00
Malic acid	12.09 ± 0.20	6.84 ± 0.40
Ethyl glucose	1.27 ± 0.03	Not reported
L-Quebrachitol	0.75 ± 0.01	Not reported
Ascorbic acid	0.47 ± 0.01	0.24 ± 0.00
Quinic acid	5.49 ± 0.16	10.89 ± 0.08

5.2 Success of fermentation

5.2.1 Non-acclimated cells

At the natural pH, the only strain able to fully convert all the malic acid to lactic acid in SBJ was the strain DSM 10492 (**I**). However, in later work, none of the tested strains were able to effectively ferment SBJ under similar circumstances (**II**). Differences in the study settings in **Study I** and **Study II** were sample volume (100 and 30 mL, respectively), inoculation level (higher inoculation level in **II**), and use of manganese salts in the growth medium (no salts included in the medium in **Study I**).

As Mn^{2+} protects *L. plantarum* from oxidative stress, lack of Mn^{2+} in the medium in **Study I** was as a potential stress factor for *L. plantarum*, leading to induced expression of Mn^{2+} starvation related genes. Mn^{2+} starvation was shown to promote morphological and membrane fatty acid changes in *L. plantarum* [273]; content of cell wall unsaturated fatty acids and lactobacillic acid were increased, similar to changes under an acid shock. Thus, lack of Mn^{2+} in the growth medium potentially worked as an inadvertent acclimation factor to improve fermentation in sea buckthorn juice. However, this would require confirmation in a separate study.

Unlike in the fermentation of sea buckthorn juice, high rate of malolactic conversion in the chokeberry juice was observed by all the tested strains, among which the strain DSM 20174 showed the highest conversion (100% after 72 h). Better success in chokeberry juice fermentation in comparison to the fermentation of sea buckthorn juice was most likely due to the higher natural pH of CB (3.5) in comparison to SB (2.7) (**I**).

Treatment with pectinolytic enzymes significantly reduced the malolactic conversion by 11.7% in samples fermented with the strain DSM 20174 in the SBJ. In contrast, fermentation of the enzyme treated SBJ with the strain DSM 100813 resulted to a significantly higher malolactic conversion compared to metabolic activity in the fresh juice (without enzyme treatment). Treatment with the pectinolytic enzyme systematically and significantly reduced malolactic conversion in CB, however, the difference varied greatly between strains. The strain DSM 20174 suffered a reduction of 1.3% in malolactic conversion rate when mash wash enzyme-treated prior to the juice extraction, while with the strain DSM 16365 this reduction was up to 38.8% (**I**).

Fermentation of lingonberry was not successful in this work, with only a trace level of lactic acid detected after the fermentation (**I**). In another instance, the pH of lingonberry juice was increased to 5.0 to allow fermentation with *L. plantarum*, as benzoic acid loses its antimicrobial activity when deprotonated [211].

5.2.2 Acclimated cells

Acclimation of *L. plantarum* cells prior the fermentation allowed fermentation of the SB juice with natural pH (2.7) with all the tested strains. Moreover, excluding the strain DSM 1055, the conversion was almost completed already within 36 hours of fermentation. Exposure to a reduced pH (4.5) and L-malic acid before the fermentation likely induced expression of genes related to the stress tolerance, leading to a better adaptation to the SB juice (**II**, **III**).

5.3 Changes in sugars and acids

5.3.1 Sea buckthorn juice

Besides the conversion of malic acid to lactic acid, statistically significant increase (4.0–7.4%) of ethyl glucose was observed in the SB samples fermented with the strains DSM 20174 and DSM 10492. Additionally, a decrease in quinic acid along with an increase in shikimic acid was detected in the SB juice fermented with the strains DSM 10492 and DSM 100813. In later work, a formation of shikimic acid, assumingly from quinic acid, was detected in all the juices where fermentation was successful (**Fig. 19**) (**III**). A second quinic acid metabolite, 3,4,5-trihydroxycyclohexane-1-carboxylic acid, was also detected (**III**). This compound was mainly formed by the strain DSM 13273. It was observed that the quinic acid metabolism was more effective at pH 3.5 compared to pH 2.7 (**III**). The strain DSM 1055 also produced succinic acid up to 0.70 mM (**III**) through the partial citric acid cycle of *L. plantarum* where succinic acid is the final downstream metabolite (**Fig. 20**). It was concluded that both the quinic acid metabolism and succinic acid formation pathways were activated to consume excess NADH from acetate production (**III**).

Regarding the metabolism of sugars, fermentation of fructose and glucose was limited at the natural pH of sea buckthorn juice (**I**, **III**), however, sugar utilization was increased at pH 3.50 (**III**). Interestingly, the strains DSM 20174 and DSM 100813 preferred fructose while other tested strains fermented mainly glucose (**Fig. 20**) (**III**). While sorbitol from chokeberries was utilized by *L. plantarum* (**I**), there was no sign of utilization of sugar alcohol L-quebrachitol from sea buckthorn juice (**I**, **III**).

Formation of disaccharide trehalose was observed during the fermentation of sea buckthorn juice with *L. plantarum*. Highest producer was the strain DSM 10492, which produced trehalose up to 0.13 mM (**Fig. 20**). In addition, the strain DSM 20174 further converted trehalose to maltose (**III**). Both trehalose and maltose are considered as multi-stress protectors in bacteria, as they can stabilize the cell wall under ethanol, acid, and osmotic stress due to the high-water holding capacity.

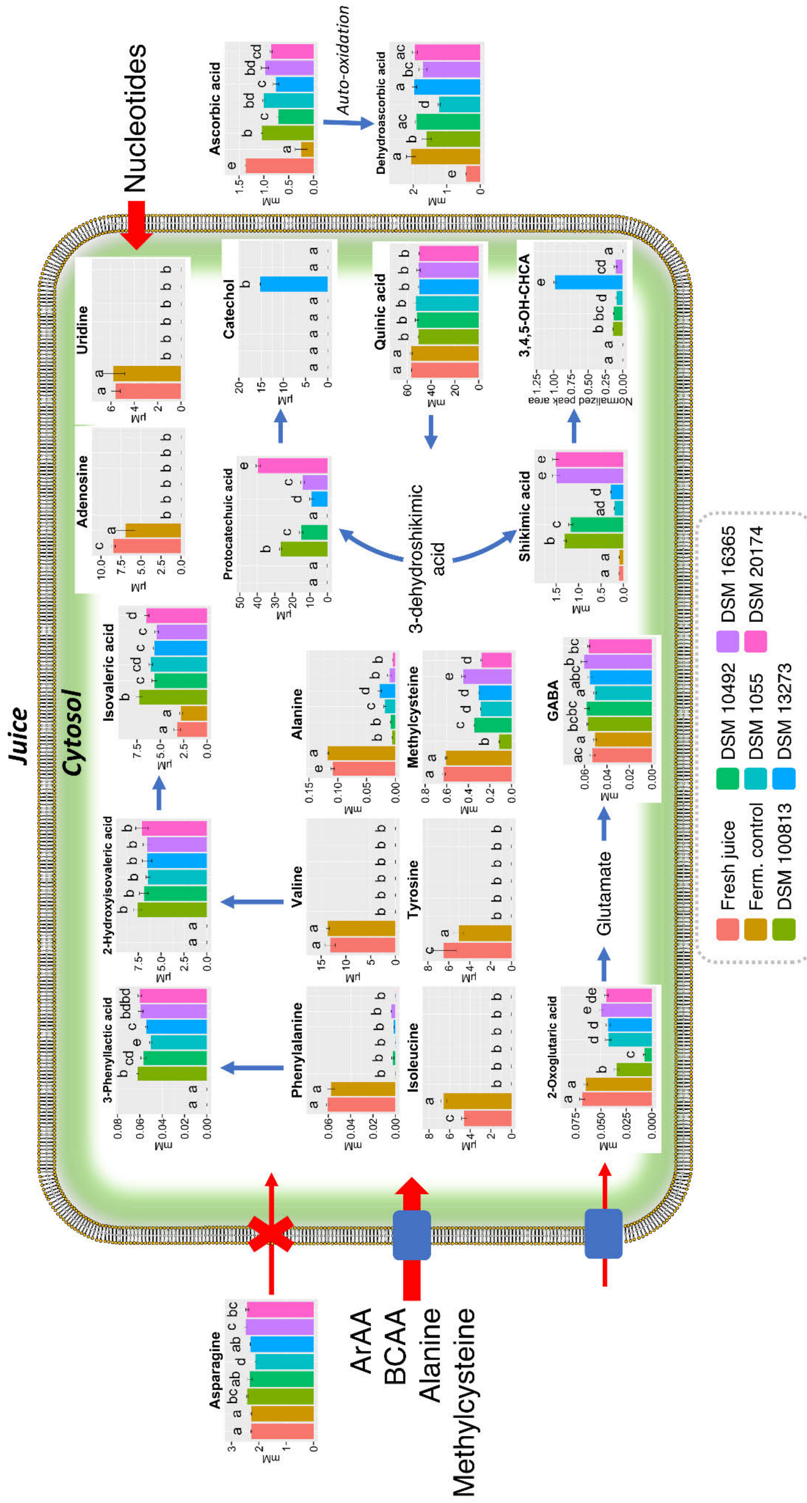


Fig. 19. Amino acid, nucleotide, and quinic acid metabolism pathways activated in *L. plantarum* during the fermentation of sea buckthorn juice. The bar plots represent concentrations (µM or mM) or normalized peak areas. Letters a–e mark a statistically significant difference over different juices with one-way ANOVA and Tukey's HSD test of significance ($p < 0.05$). Results are average ± standard deviation.

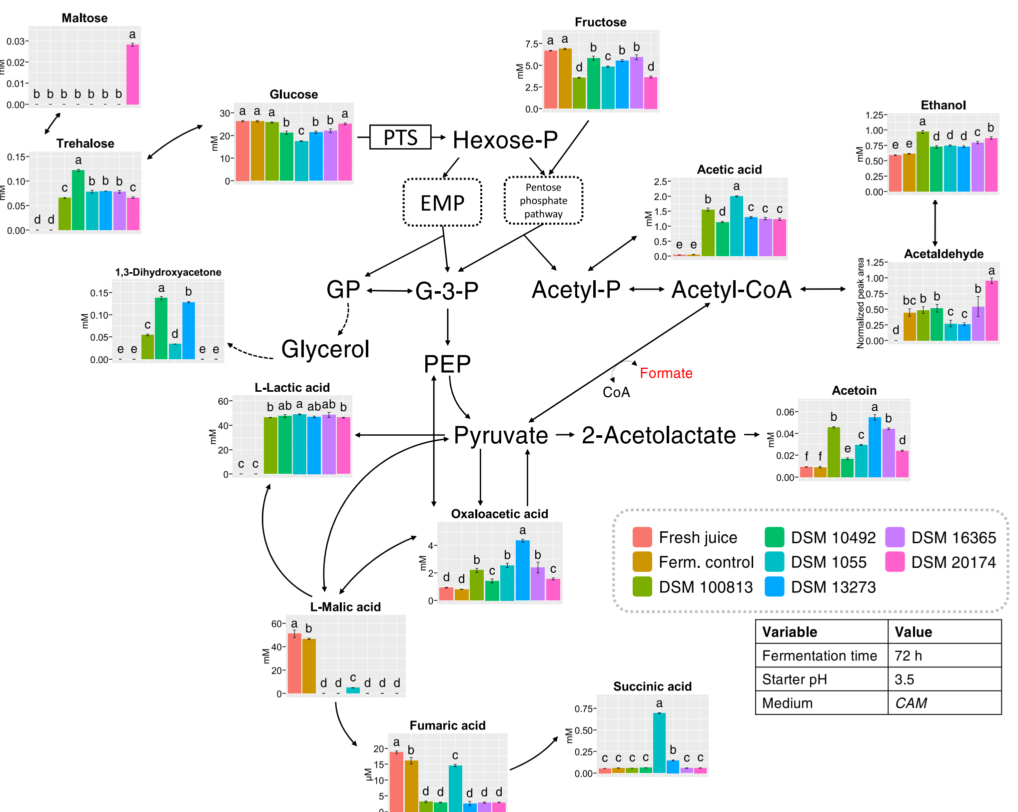


Fig. 20. Homo- and heterofermentative pathways activated in *L. plantarum* during the fermentation of sea buckthorn juice. The bar plots represent concentrations (µM or mM) or normalized peak areas. Letters a–f mark a statistically significant difference over the different juices with one-way ANOVA and Tukey's HSD test of significance ($p < 0.05$). Results are average ± standard deviation (**III**).

Therefore, it was concluded that *L. plantarum* produced these compounds as a response to acid stress [274]. While no established pathway in *L. plantarum* currently exist, it was assumed based on the existing knowledge on trehalose biosynthesis in other microorganisms that the precursors were D-glucose and UDP-glucose [274]. Recently, accumulation of trehalose by *L. plantarum* was reported under ethanol stress [275].

Study by Tkacz et al. [188] had a high similarity with the study setting in our work (**I**), as the same strains were used to ferment SBJ without pH adjustment. In their report, level of MLF was only moderate, and the most effective fermentation was by the strain DSM 20174 (21% malolactic conversion, 0.1 unit increase in pH). In the same study, also a mix of sea buckthorn and apple juice was fermented, which proved to be a more suitable fermentation matrix, showing an additional approach for improving fermentation besides acclimation or pH adjustment.

SBJs inoculated with *L. plantarum* had a significantly higher amount of ascorbic acid after fermentation compared to the juices incubated without inoculation. Preservation of ascorbic acid by lactic acid fermentation was reported earlier by Filannino et al. [187]. This work and study from Tkacz et al. [188] together suggest that retaining of the antioxidant compounds (i.e. anthocyanins and ascorbic acid) during fermentation in berry materials is due to the increased antioxidant capacity. However, at the same time, in **Study I**, the strain DSM 10492 had the most dynamic metabolism of phenolic compounds out of all the tested strains yet showed the lowest protection of ascorbic acid and anthocyanins from oxidation. This is contradictory to the assumption that the lactic acid bacteria modify the antioxidant capacity of plant materials though phenolic metabolism and suggests that there are other underlying mechanisms that play a role.

5.3.2 Chokeberry juice

The concentrations of total sugars and fructose of the chokeberry juice were significantly reduced in all fermented chokeberry samples by 6.1–13.7% and 3.9–17.6%, respectively, while glucose and sorbitol were reduced in a varying degree depending on the material and the strain (4.1–13.6% and 0.5–10.5%, respectively). Like with SBJ, quinic acid was converted to shikimic acid also during the fermentation of chokeberry juice, and the content of the latter was increased from 0.05 to 0.11 g/L (**I**).

5.4 Phenolic profile of the berry juices

5.4.1 Chokeberry

Anthocyanins in the chokeberry juices were primarily cyanidin glycosides with traces of pelargonidin derivatives. The most abundant anthocyanin compounds detected were in a descending order of abundance cyanidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-xyloside, and cyanidin-3-*O*-rhamnoside. Chlorogenic acid and neochlorogenic were the main hydroxycinnamic acids detected from the juice with smaller amounts of 3-*O*-*p*-coumaroylquinic acid and caffeic acid also detected. The main flavonol compounds were in descending order were quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-rhamnoside-hexoside, quercetin-3-*O*-rutinoside, and quercetin-3-*O*-vicianoside. The main hydroxybenzoic acid was protocatechuic acid. The phenolic profile of the CB juice was similar to earlier reports [254,256].

5.4.2 Sea buckthorn

In the ethyl acetate extracts of SBJ, the most abundant group of phenolic compounds were flavonols. The major flavonol glycosides were isorhamnetin-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucose-7-*O*-rhamnoside and quercetin-3-*O*-glucoside, which constituted 78% out of the total flavonols. Regarding the minor flavonol compounds, two acylated isorhamnetin derivatives with a substitution group of *m/z* value 84 were detected. Earlier, a isorhamnetin derivative with acyl group of a quasimolecular ion of the same *m/z* value was identified in sea buckthorn berries [244]. From the class of hydroxybenzoic acids, protocatechuic acid and a gallic acid derivative were detected. While no other common phenolic acids (ferulic, *p*-coumaric or caffeic acid) was detected from SBJ, majority of these compounds are present as bound compounds (glycosides and esters) in the sea buckthorn berry [245] and were likely not detected with the methods used in the study (**I**).

5.4.3 Effect of enzyme treatment on phenolic profile

Pectinolytic enzyme treatment often increases content of phenolic compounds due the release of bound phenolics from the cell wall matrices and polysaccharides [276]. In this work, enzyme treatment significantly increased the total contents of anthocyanins, hydroxybenzoic acids, and hydroxycinnamic acids in the CB juice.

In SBJ, enzyme treatment significantly increased the content of hydroxybenzoic acids and flavonols. Additionally, enzyme treatment increased the flavonol aglycone content in both SB and CB juices. Enzyme-treated SBJ had a lower amount of isorhamentin-3-*O*-glucoside-7-*O*-rhamnoside and isorhamnetin-3-*O*-

rutinoside, but a higher amount of isorhamnetin-3-*O*-glucoside compared to the fresh juice. This result suggested that the enzyme used in the study possessed an undeclared rhamnosidase activity, which led to formation of flavonol aglycones during the incubation period (**I**).

5.4.4 Changes in anthocyanins during fermentation

The total anthocyanin content was mostly affected by pasteurization, leading to a reduction from 37.97 ± 0.41 to 30.9 ± 0.09 mg/100 mL in the fresh juice and from 56.74 ± 0.32 to 37.14 ± 0.48 mg/100 mL in the enzyme-treated juice. Anthocyanin content continued to decrease during fermentation, to the levels of 20.93 ± 0.13 and 29.77 ± 0.18 mg/100 mL in fresh juice and enzyme-treated juice, respectively. However, after the fermentation, the samples inoculated with bacteria had a significantly higher anthocyanin content (except for the DSM 10492) than the control juice without a bacterial inoculation, pointing to a possible anthocyanin stabilizing factor related to *L. plantarum* (**I**).

5.4.5 Changes in flavonols during fermentation

In the chokeberry juice, the strain DSM 10492 significantly decreased the total content of flavonol glycosides in the fresh juice samples (i.e., no enzyme treatment), while fermentation with the strains DSM 100813 and DSM 10492 showed significant reduction in the flavonol content in enzyme-treated juices. Fermentation with the strain DSM 10492 reduced the content of flavonols by 9–14% in CB juice. No metabolic products from the flavonol glycoside metabolism were detected (**I**).

However, in sea buckthorn, there was no statistically significant difference in the total flavonol content, or in the contents of individual flavonol compounds, between different treatments (**I**). The different glycosidases produced by *L. plantarum* can be considered the main enzymes for flavonol glycoside modification. Therefore, reasons for the lack of flavonol modification in SBJ can be speculated to be several. First, lack of affinity of *L. plantarum* glycosidases with isorhamnetin derivatives present in the SBJ. However, formation of the isorhamnetin aglycone was reported in the fermentation of cactus cladode pulps [187], meaning that *L. plantarum* has also shown enzymatic activity with isorhamnetin glycosides. A second potential explanation is the low pH of SBJ which has either reduced the gene expression of glycosidic enzymes, or has inhibited the enzymatic activity, or both. Earlier, a low pH (3.2) was reported to reduce the β-glucosidase activity of *L. plantarum* in model wines [105]. A third explanation is that the presence of glucose has downregulated the expression of β-glucosidase or any of the other glycosidase related genes [104]. In a material opposite of SBJ in terms of pH and sugar content, fermentation of silkworm thorn

leaf infusion with *L. plantarum* yielded high amounts of quercetin from quercetin-7-*O*-glucoside, and kaempferol from kaempferol-3-*O*-glucoside and kaempferol-7-*O*-glucoside [277]. Plant leaves infusions typically have low acidity, a low sugar content, and a high phenolic content. Absence of readily available carbon sources could have increased the expression of glucosidase genes, leading to an effective conversion of flavonol glycosides to the corresponding aglycones. In this context, flavonols were largely unaffected in this work possibly due to the factors that inhibited the expression of glycosidases required for the flavonol glycoside modification.

5.4.6 Changes in phenolic acid content

Fermentation with the strain DSM 10492 reduced the content of caffeoylquinic acids and other hydroxycinnamic acids by 20–24% in the chokeberry juice. However, there was no significant increase in the contents of either caffeic acid or quinic acid, as the compounds were metabolized further by *L. plantarum* (**I**). While quinic acid metabolism was later investigated (**III**), the metabolic product of caffeic acid in fermentation of chokeberries was not discovered. If caffeic acid was reduced to dihydrocaffeic acid [194], the loss of side chain double bond caused absorbance maximum to shift from 325 nm to 206 nm, meaning that the compound could have not been detected with the photodiodearray detector which was used in **Study I**.

So far, no esterase enzyme has been discovered from *L. plantarum* that is effective in hydrolyzing caffeoylquinic acids (**Section 2.4.4**). However, it is still plausible that the esterases identified earlier from *L. plantarum* were responsible for the observed reduction in chlorogenic acid content in the fermentation of chokeberry juice (**I**). For example, feroyl esterase Lp_0796 showed a mild hydrolytic activity on chlorogenic acid [76,79]. However, this gene is commonly present in *L. plantarum* and thus does not explain the strain-dependent difference in the chlorogenic acid metabolism (**I**). Therefore, it is possible that the strain DSM 10492 produced an unknown esterase with activity on caffeic acid esters.

While the flavonols in SBJ were unaffected by fermentation (**I**), increase in the protocatechuic acid content was detected in SBJ (**I**, **III**). It was concluded that the increase in protocatechuic content acid was due to quinic acid metabolism. Furthermore, the strain DSM 13273 decarboxylated protocatechuic acid into catechol (**III**). Unlike the metabolism of quinic acid into 3,4,5-trihydroxycyclohexane-1-carboxylic acid through the so-called *reductive pathway*, which allows recovery of cofactor NAD^+ [60], the metabolic benefit of conversion of quinic acid into protocatechuic acid and catechol is less clear. Obvious benefit would be a deacidification effect, as protocatechuic acid is a weaker acid than quinic acid (pK_a 4.48 and 3.46, respectively), and decarboxylation to catechol removes a proton donating group altogether.

5.5 Volatile compounds

5.5.1 Volatile profile of fresh sea buckthorn juice (II)

In total, 90 volatile compounds were identified from the fresh sea buckthorn juice, of which were 53 esters, 7 acids, 6 alcohols, 7 aldehydes, 3 alkenes, 8 ketones, 4 terpenes and 3 sulfur-containing compounds. Non-branched, branched, and aromatic esters were detected. Identified non-branched fatty acid esters with varying acyl carbon numbers were, in descending order of abundance, C6, C8, C3, C10, C5, C7 and C2. The most abundant branched esters were those with acyl group of 3-methylbutanoates, 2-methylbutanoates, 2-methylpropanoates, or 3-methyl-2-butenoates. Esters of benzoate were the main aromatic esters.

The most abundant compounds in the GC-MS chromatograms were, in descending order, 3-methylbutyl 3-methylbutanoate, 3-methylbutyl hexanoate and ethyl hexanoate. A majority of the tentatively identified esters have fruity odor descriptor, while esters and terpenes with floral odor descriptor were also detected.

The main volatile acids detected were acetic acid and medium-chain fatty acids (C6-C9), while fatty-acid derived aldehydes with the same carbon numbers were also detected. Other aldehydes detected were benzaldehyde and acetaldehyde. Fatty acid derived ketones with acyl chain lengths of 3, 4, 5, 7, 9, and 11 were detected. However, except for ethanol and 1-heptanol, no corresponding alcohols to aldehydes or ketones were detected.

In earlier reports it has been observed that depending on the cultivar, subspecies, growth location and growth season, the main volatile compounds in sea buckthorn juice are ethyl 3-methylbutanoate, ethyl hexanoate, and 3-methylbutyl 3-methylbutanoate at varying ratios (**Fig. 19**). Therefore, the volatile compound profile reported in **Study II** was in accordance with earlier reports. Based on the structures of volatile esters present in the sea buckthorn berry, the precursors are likely derived from the same precursor pool (**Fig. 20**), however, further studies are required to identify the cellular mechanisms that control substrate availability in the ester biosynthesis.

5.5.2 Changes in volatile profile of sea buckthorn juice during fermentation (II)

Incubation decreased the content of esters and terpenes, whereas fermentation increased the levels of volatile acids, ketones, and alcohols. Moreover, fermentation lowered the content of fatty acid-derived aldehydes. Juices fermented with the strain DSM 1055 had the highest acid and alcohol content while fermentation with the strain DSM 13273 resulted to the highest content of ketones (**Fig. 21**). Compared to inoculation with other strains, fermentation with

the strains DSM 16365 and DSM 100813 resulted to a rapid MLF, formation of less volatile acids, and lower loss of esters and terpenes, important for natural sea buckthorn flavor.

In principal component analysis, fermented samples were associated with 3-methyl-1-butanol (fermented aroma), ethanol, and benzyl alcohol (floral). 3-Methyl-1-butanol was possible derived from leucine metabolism (**Fig. 12**) and/or ester hydrolysis. As the phenylalanine metabolism by *L. plantarum* was detected in sea buckthorn juice in **Study III**, it can be speculated that phenylalanine was the precursor for benzyl alcohol detected from fermented SBJ (**II**). As discussed earlier, phenylpyruvate, derived from the transamination of phenylalanine, can be non-enzymatically converted to benzaldehyde when exposed to the Mn^{2+} reservoir of *L. plantarum* (**Fig. 12**). Benzaldehyde can then be reduced to benzyl alcohol by the benzyl alcohol dehydrogenase (Lp_3054) (**Fig. 11**). Ethanol was likely from the heterofermentative pathway of *L. plantarum* (**II**).

Increase in the volatile acid content was due to the production of acetic acid, 3-methylbutanoic acid (cheesy aroma) and medium chain fatty acids (fatty and cheesy aromas). As acetic acid is one of the end-products in the mixed acid fermentation pathways, it is a common metabolite of *L. plantarum*. 3-Methylbutanoic acid was likely derived from same precursos as 3-methyl-1-butanol, meaning from leucine metabolism and ester hydrolysis (**Fig. 12**) (**II**).

Increase in the volatile ketone content especially in juices fermented with the strain DSM 13273 (**Fig. 21**) was due to the increase in buttery ketones acetoin and diacetyl contents. In accordance with earlier studies, the main ketone product was acetoin. 2-Undecanone (fruity aroma) was positively correlated with fermented samples in PCA models, however, precursor for this volatile was not established. No volatile phenols (e.g. 4-ethyl phenol) were detected in any of the juices (**II**).

Whether the changes in volatile profiles in **Study II** were beneficial for overall aroma of sea buckthorn juice is not clear. While the content of several floral volatiles was increased, at the same time, increase in volatile acidity (i.e., potential off-aromas) was observed. Additionally, loss of the fruity esters during fermentation could have negative impact on sensory value of SBJ. Due to large number of samples the juice volume per sample was relatively low (30 mL), which had a high surface-to-volume ratio. Therefore, it is possible that at larger volumes, let alone on an industrial scale, the loss of volatiles during fermentation could be significantly lower compared to the results reported in **Study II**.

No report apart from this work exist studying change in the volatile profile of SBJ after fermentation with *L. plantarum*. However, Tiitinen et al. [278] studied the impact of MLF with *O. oeni* on SBJ volatile profile. While the content of various esters was decreased similary to **Study II**, increase in concentrations of ethyl acetate, 3-methylbutyl acetate, and ethyl butanoate were detected.

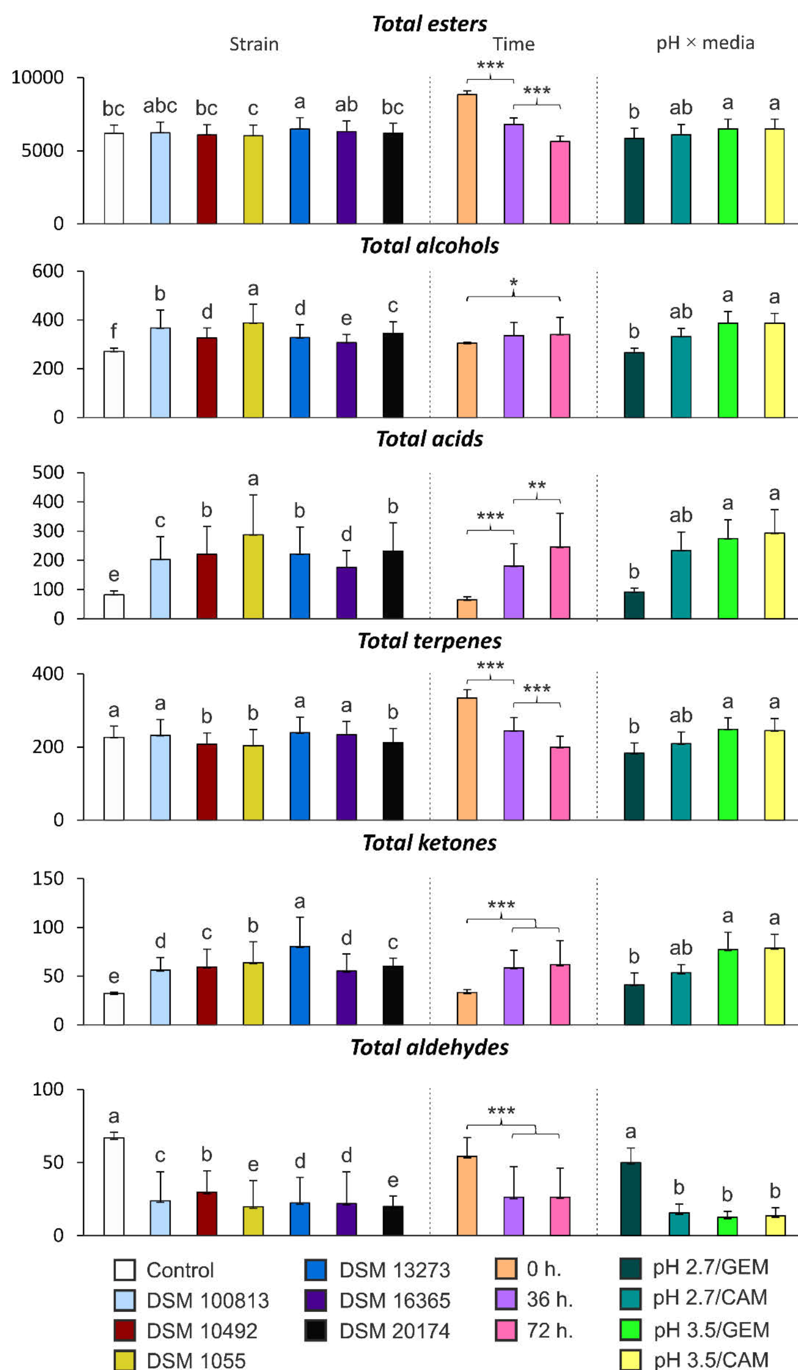


Fig. 21. Sums of the volatile compound subgroups over different fermentation variables. Results are mean ± standard deviation. Asterisks mark the groups that are statistically different (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Tukey's HSD test of significance was used for the comparisons. Y-axis represents semi-quantified volatile content (µg/L).

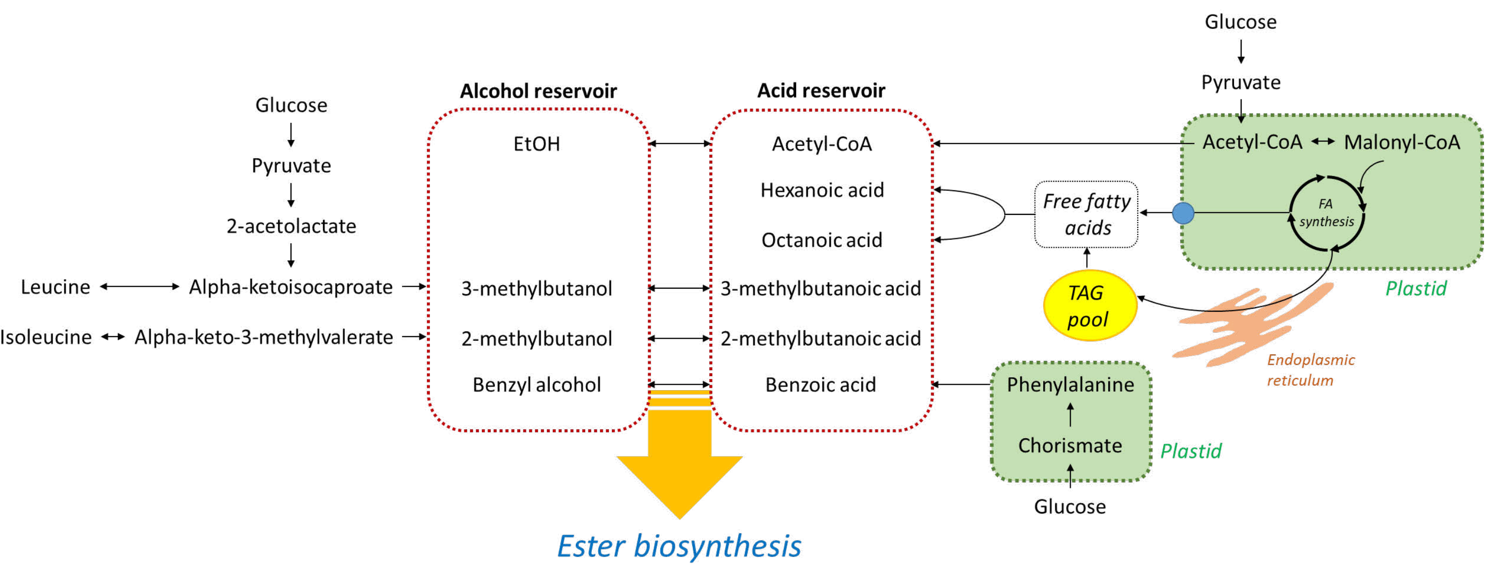


Fig. 22. Suggested ester biosynthesis reservoirs in the sea buckthorn berry. Adapted from Dudareva et al. [279].

Additionally, ethanol and 3-methyl-1-butanol contents were increased, which was also reported in **II**.

All in all, as the odor thresholds of aroma-active volatile compounds vary significantly between compounds and are strongly dependent on the sample matrix, further studies are required to determine how MLF affects the aroma and sensory properties of fermented SBJs.

5.6 Changes in other metabolites

From the 1D-NOESY spectra, several metabolites not yet discussed and not detected in either **Study I** or **Study II** were identified from the fresh sea buckthorn juice (**III)**. These included free amino acids (methylcysteine, valine, isoleucine, alanine, tyrosine, asparagine, and phenylalanine), nucleotides (adenosine, uridine), succinic acid, 2-oxoglutaric acid, choline, fumaric acid, formic acid, trigonelline, ribose, and methyl glucoside. From all identified metabolites, trigonelline, asparagine, ethyl glucose, *myo*-inositol, and L-quebrachitol showed no significant change after fermentation in any of the samples (**III**).

During fermentation, *L. plantarum* consumed most of the free amino acids, especially branched-chain amino acids and phenylalanine. Amino acid catabolism was more pronounced at higher pH. However, asparagine present in SBJ was not utilized by *L. plantarum* (**III**).

One sulfur-containing amino acid was detected from SBJ (methylcysteine), which was also absorbed by *L. plantarum* (**Fig. 19**) (**III**). While methionine is a growth limiting amino acid for *L. plantarum*, previous studies have suggested that the species is capable to convert cysteine into methionine (**Section 2.6.2**). However, it was not confirmed in this doctoral thesis whether *L. plantarum* absorbed methylcysteine only due to the structural similarity to cysteine and methionine, or if it was able to utilize methylcysteine in its metabolism.

The main metabolite of phenylalanine was 3-phenyllatic acid, produced from transamination *via* phenylpyruvate. In the first step, the amino group from phenylalanine is transferred to 2-oxoglutaric acid, yielding glutamic acid, an essential amino acid for *L. plantarum*. Therefore, it was an unexpected result that 2-oxoglutaric acid present in SBJ was only partly absorbed by *L. plantarum* during fermentation (**III**). The only strain that effectively absorbed 2-oxoglutaric acid was the DSM 10492 (**Fig. 19**). Whether the the limited uptake in other strains was controlled (by downregulation of uptake related genes) or not (e.g., uptake inhibited due to the acidic conditions) requires further studies. Moreover, GABA, derived from decarboxylation of glutamic acid, was detected in juices fermented with strain DSM 1055. Higher yield of GABA was detected at pH 2.7

compared to pH 3.5, suggesting that the compound was produced to counter the acid shock (**III**).

The strains DSM 10492 and DSM 13273 produced significantly higher content of 1,3-dihydroxyacetone (DHA) (0.13–0.14 mM) compared to the other strains (0–0.05 mM) (**Fig. 20**). In the case of strains DSM 100813 and DSM 1055, DHA was produced solely by cells grown in the *CAM* while little to no production was detected by cells grown in the *GEM*. As the *CAM*-grown cells were adapted to the acid stress prior to inoculation, it can be speculated that DHA production was related to cellular stress. Earlier, DHA was detected from fermented vegetable juice [280]. One potential source for DHA is from the oxidation of glycerol with NAD^+ by the enzyme glycerol dehydrogenase (encoded by *gldA*). While no copy of *gldA* gene from *L. plantarum* was reported by Doi [281], UniProt query provided a putative *gldA* gene for *L. plantarum* based on a sequence homology (accession N692_08665). Rivaldi et al. reported that the main pathway in glycerol metabolism in *L. plantarum* under physiological conditions was formation of glycerol-phosphate by glycerol kinase while very little glycerol oxidation was observed [282]. However, further studies are required to study if metabolic flux is shifted to glycerol oxidation under acid shock, which would explain DHA accumulation during fermentation of SBJ.

5.7 Methological considerations and limitations of the study

While this work focused on optimizing multiple fermentation variables (pH, fermentation time, strain, medium composition), there are numerous other factors to be considered in the product development of fermented plant-based foods (**Fig. 23**). One factor not considered in this work was "optimizing" the origin of the raw material. The chemical composition of a plant material can be substantially different depending on the subspecies, cultivar, growth location, and growth year. As it was discussed in this thesis, there are studies showing that the fermentation end-product can be significantly different based on the cultivar of the raw material alone [197,283].

Another factor not considered in this work was optimizing the inoculation rate since the initial cell count can affect the overall metabolic activity due to quorum sensing. This work also did not analyze if the cell number of *L. plantarum* decreased, was maintained, or increased during fermentation of sea buckthorn or chokeberries. This analysis was left out due to the high number of samples. Many studies related to the fermentation of fruit or berry materials have included a storage trial after fermentation, which was missing in this work. Future work should analyze survival of *L. plantarum* in berry juices both during fermentation and storage. Storage trial would also allow determination of the residual

metabolic activity of *L. plantarum* in sea buckthorn or other similar materials at low temperature.

Only little phenolic metabolism was detected sea buckthorn juice (**I**), and thus this thesis work could be extended by studying whether sea buckthorn flavonol glycosides are metabolized more effectively at elevated pH or after acclimation. It was assumed that the glycosidic enzymes of *L. plantarum* were inhibited due to presence of sugars and low pH in SBJ, which was the reason for the limited metabolism of flavonol glycosides. However, further studies would be required to confirm if this was the case.

Only SBJ was used in **Studies II** and **III**, but chokeberry juice was also a promising matrix for malolactic fermentation with *L. plantarum* (**I**). As chokeberry has a very high phenolic content and subsequent low consumer value due to the intense bitterness and astringency, further studies to modify the chokeberry phenolic profile more effectively than in this work could be worthwhile. Also, one of the key odor compounds in chokeberry juice is benzaldehyde, making it a potential target to improve sensory value by reducing this aldehyde to a floral benzyl alcohol with lactic acid bacteria.

Multiple conclusions in this thesis rely on the assumption that fermentation of sea buckthorn and chokeberry juices with *L. plantarum* in general improved the antioxidant capacity. However, no *in vitro* antioxidant capacity analysis was performed in this thesis, and thus it would be necessary to confirm the assumption that protection of ascorbic acid and anthocyanins from oxidation during fermentation was due to the increased antioxidant capacity. Also, as an increase in antimicrobial compounds (lactic acid, acetic acid, and 3-phenyllactic acid) was reported in SBJ after the fermentation (**III**), antimicrobial trials would be needed to test if the accumulation of these compounds had any significant impact on the microbial stability of SBJ.

Analysis of the volatile compounds in fermented sea buckthorn did not consider whether the changes in the volatile compound profile had impact on the detectable odor, and the study would have benefitted from a GC-O analysis to determine the key odorants in both the fresh and the fermented SBJ **(II**).

Ultimately, the main shortcoming of this work was lack of the sensory evaluations to confirm whether the observed chemical changes were significant enough to produce a difference in flavor, and whether the change in flavor was beneficial or not. Thus, the next step to take this research further would be to select the most promising samples for sensory and consumer trials.

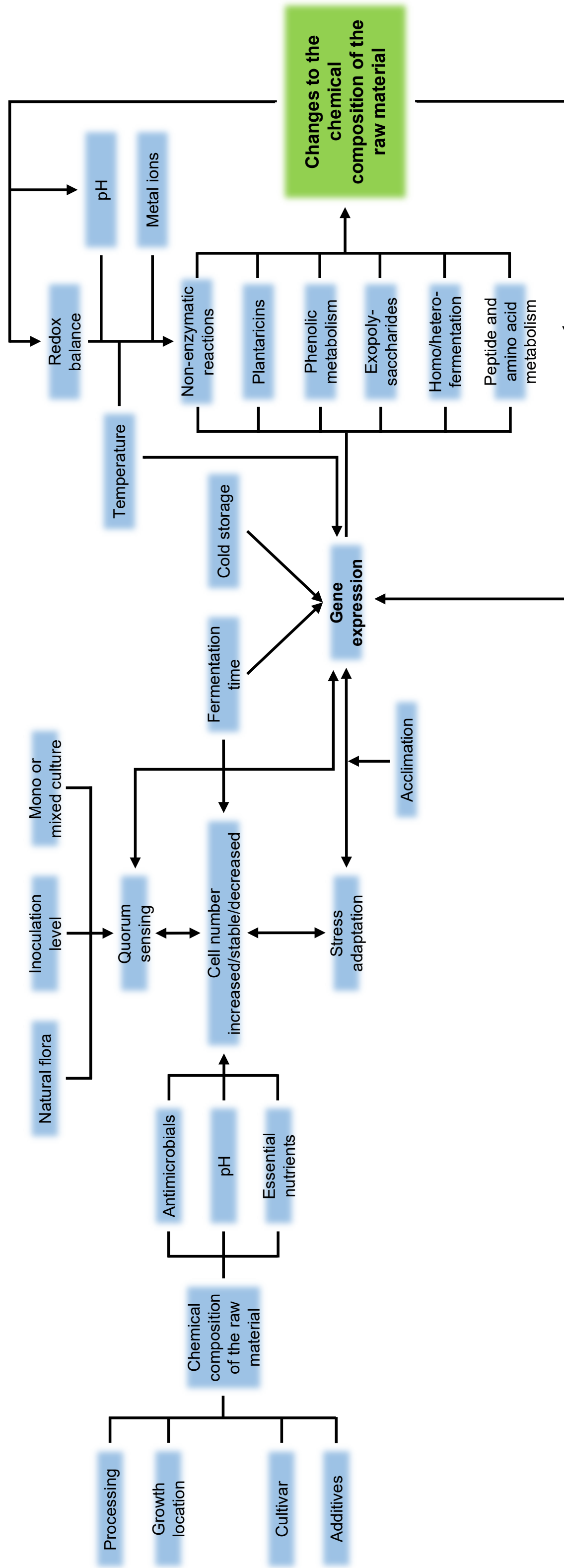


Fig. 23. Factors related to the raw material, fermentation setting, and inherent properties of the fermenting organism that play a role in development of lactic acid fermented plant-based foods.

6 SUMMARY AND CONCLUSION

This work set out to study the potential of MLF to reduce the acidity of berry materials, to use fermentation to modify bitter and astringent compounds in berries (i.e., phenolic compounds), and to determine if compounds that promote functional properties, such as storage stability, are generated during MLF Initially, it was concluded that sea buckthorn juice was the optimal material due to a very high content of malic acid and a low content of sugars. While in wine MLF pH is typically increased by 0.2 units, an overview of existing literature revealed that in most studies where *L. plantarum* is used to ferment non-alcoholic fruit material, the pH either remains unchanged or decreases. In fact, the increase in pH observed in this work (0.34 units) is among the highest, if not the highest, observed pH increase when MLF has been used for deacification. This suggests that sea buckthorn juice might be one of the few materials where utilization of this approach is applicable. While the flavonol glycosides and phenolic acids of chokeberry juice were metabolized by *L. plantarum,* there was no metabolism of flavonol glycosides present in fermentation of sea buckthorn juice. It was speculated that the low pH and the presence of sugars inhibited the β-glucosidase activity of *L. plantarum* required for the metabolism of these compounds.

Although not the initial target of the research, it was observed that certain strains protected ascorbic acid and anthocyanins from degradation during fermentation. Based on the existing literature, this was thought to be associated with an increased antioxidant capacity. Typically, this effect by *L. plantarum* has been associated with phenolic modification, however, results with the strain DSM 10492 contradicted this assumption; the strain was most effective in the metabolism of phenolic compounds but showed the least beneficial impact on the antioxidant capacity. This suggests that there are other underlying mechanisms behind the antioxidant capacity modification of *L. plantarum*.

In this work, acclimation by adding malic acid to the growth medium and decreasing the pH from 6 to 4.5 was an effective way to improve fermentation in sea buckthorn juice. The benefit of this approach was that change in the basal medium composition had no clear negative effect on the microbial growth of *L. plantarum* and the acclimation medium could be inoculated with a single colony, similar to the MRS medium. Therefore, this approach is easy to implement in both research and product development.

Increasing the juice starter pH from a natural 2.7 to 3.5 improved fermentation in a similar manner to acclimation. This increased the fermentation of sugars in sea buckthorn juice, and also allowed more complex metabolic activity during fermentation. The beneficial changes made by increasing the pH were the formation of antimicrobial compounds, an increased antioxidant capacity, and an increased metabolism of the secondary metabolites. At the same time, it was

observed that in the fermentation of sea buckthorn, *L. plantarum* utilizes several approaches to circulate redox cofactors to allow energy production from acetyl-CoA, including succinic acid and ethanol formation as well as quinic acid metabolism. Most of the observations mentioned above also showed strain-dependent variations.

When considering the research field (i.e., fermentation of plant-based materials with lactic acid bacteria) in general, there is an evident gap between genomic studies and the practical applications of *L. plantarum* in food models. One approach would be to utilize an “omics” approach, combining metabolomics, transcriptomics, proteomics etc. for a carefully selected strain and a raw material combination. Another goal would be to establish the metabolic pathways and genes that are most relevant for fruit and berry fermentations to produce materials with an enhanced flavor. As discussed in the literature review, fermentation with *L. plantarum* has improved flavor in various plant-based materials. However, the most relevant cellular functions responsible for these changes should be discovered in addition to what conditions drive the activation of these metabolic systems. This approach is already in use in wine MLF starter development, as studies often screen for the presence of specific genes or gene related activity from starter candidates, such as genes for β-glucosidase, esterase/alcohol transferase (EstA), and citrate lyase.

There is a growing interest on the exploitation of berry materials using fermentation, as highlighted by the recent review by Schubertová et al. [284] focusing on research related to fermented sea buckthorn. This doctoral thesis has provided new insight into the development of berry-based products using malolactic fermentation as a bioprocessing method. The conditions were determined that drive effective deacidification of materials such as sea buckthorn with *L. plantarum* without fermentation of sugars. In addition, the conditions that created additional benefits though potential modification of functional (i.e. antioxidant and antimicrobial) properties were also determined. Results from this thesis can be applied to product development of other materials with low pH and high malic acid content as well, including sour apples, sour cherries, and cranberries.

ACKNOWLEDGEMENTS

This thesis work was carried out at the Food Chemistry and Food Development unit, Department of Life Technologies, University of Turku. Part of the thesis work was done in collaboration with the Center of Food and Fermentation Technologies (CFFT) from Tallinn, Estonia. The NMR analyses related to this doctoral thesis were performed at the Instrument Centre (Åbo Akademi, University of Turku).

There is no doubt that this PhD project would have been possible without the support from the Doctoral Programme in Molecular Life Sciences, who funded me a full-time doctoral candidate position for two and a half years. I am also extremely grateful for the personal grants from the Raisio plc's Research Foundation, the Niemi Foundation, the Finnish Food Research Foundation, and the TOP foundation. In addition, I extend my gratitude to the Finnish Cultural Foundation, the OLVI foundation, and the Magnus Ehrnrooth foundation for the financial support that have allowed several visits to both domestic and international conferences and have helped me to cover the research expenses. Finally, I thank Agronomiliitto for the grant to cover expenses related to proofreading and printing of this thesis.

Baoru and Oskar, thank you for being my valued supervisors, teachers, mentors. I have always been able to count on your support and expertise, and your insight have helped me to improve my work to its best potential. You have taught me a great deal about food science, but at the same time, you have appreciated and listened to my ideas.

During my stay in Estonia in 2016, I was happy to get to know people from the CFFT. I want to thank the whole staff of CFFT who made me feel welcome and made my stay in Estonia a pleasant experience. Furthermore, I would like to thank Rain Kuldjärv, Ranno Nahku, and Raivo Vilu for our scientific collaboration. Related to this work, I extend my gratitude to ldar Nisamedtinov, Sibylle Krieger-Weber, and to the Lallemand Inc. for providing cell cultures.

I deeply appreciate scientific collaboration with my co-authors PhD Johanna Jokioja, PhD Maaria Kortesniemi, and PhD Raghunath Pariyani. I couldn't have managed without your expertise. I wish to thank my streering group, prof. Tuomo Sainio, PhD Jari Sinkkonen, and PhD Jukka-Pekka Suomela. Your feedback and encouraging words have pushed my work forward. For reviewing this thesis, I want to thank prof. Giuseppe Spano and prof. George-John Nychas for their insight and feedback. I wish to thank Elizabeth Nyman for proofreading of this thesis.

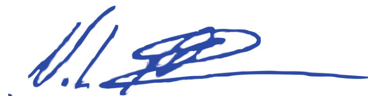
I greatly appreciate Gabriele Beltrame, Niina Kelanne, Ye Tian, Natasa Mantziari, and Heikki Aisala for sharing this journey with me both during and after working hours. In addition, I want to acknowledge Amruta Kulkarni, Anna

Puganen, Annelie Damerau, Eija Ahonen, Ella Aitta, Heikki Kallio, Kaisa Linderborg, Kang Chen, Kati Hanhineva, Liz Gutierrez Quequezana, Marika Kalpio, Marko Tarvainen, Mikael Fabritius, Moona Rahikainen, Oluwaseun Samson, Salla Laito, Sari Mustonen, Shuxun Liu, Tanja Kakko, Wei Yang, Wenjia He, and Xueying Ma for being a valued collague and more. I thank Sarianna Koivula for her help in the laboratory work. I want to also thank Anu Hirvensalo and Heli Kuusela for both our discussions during coffee breaks and their supportive work in our unit.

For all the people in Kultapossukerho, Team Hammock, and New Pascas, I want to thank you for being my valued friends. Friends are like research topics: you must choose them carefully. I think I have succeeded in this.

Kiitos isälle ja äidille, jotka ovat kannustaneet yrittämään parhaani jo ala-asteelta lähtien. Kiitos sisaruksilleni kaikista yhteisistä hetkistä joulupöydän tai mökkilaiturin ääressä. Olette olleet minulle mittaamaton voimavara.

Finally, there are no words to measure how important you, Saana, have been to this journey to share both the good and the not-so-good moments with me. There are many endeavors I wouldn't have undertaken if you weren't there to encourage me, including this PhD. Thank you for your love and support.



Turku, September 2021

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APPENDIX: Supplementary materials

Supplementary Table S1. List of the genes and enzymes identified from *L. plantarum* referenced in this work.

Name in figure	**Enzyme full name**	**Gene (Locus name, Uniprot Entry)**	**EC**	**Strain**	**Ref.**
Figure 3					
Ack	Acetate kinase	*ack1* (*lp_0210*, F9UT17), *ack2* (*lp_0310*, F9UTR4), *ack3* (*lp_2242*, Q88V41)	2.7.2.1	WCFS1	[41,42,285]
AlcDH	Bifunctional protein: alcohol dehydrogenase; acetaldehyde dehydrogenase	*adhE* (*lp_3662*, F9ULK9)	1.1.1.1 / 1.2.1.10	WCFS1	[41,42,285]
	Alcohol dehydrogenase	*adh1* (*lp_1665*, F9UP30), *adh2* (*lp_2873*, F9URX3)	1.1.1.1	WCFS1	[41,42,285]
AldDH	Bifunctional protein: alcohol dehydrogenase; acetaldehyde dehydrogenase	*adhE* (*lp_3662*, F9ULK9)	1.1.1.1 / 1.2.1.10	WCFS1	[41,42,285]
	Acetaldehyde dehydrogenase	*acdH* (*lp_0329*, F9UTT1)	1.2.1.10	WCFS1	[41,42,285]
Ald	α-Acetolactate decarboxylase	*aldB* (*lp_2030*, F9UPY7)	4.1.1.5	WCFS1	[41,42,285]
Als	α-Acetolactate synthase	*als* (*lp_1005*, F9UMJ0)	2.2.1.6	WCFS1	[41,42,285]
Cit	Citrate lyase, γ-subunit	*citD* (*lp_1107*, Q88XS8)		WCFS1	[41,42,285]
	Citrate lyase, β-subunit	*citE* (*lp_1108*, F9UMR9)	4.1.3.34	WCFS1	[41,42,285]
	Citrate lyase, α-subunit	*citF* (*lp_1109*, F9UMS0)	2.8.3.10	WCFS1	[41,42,285]
Frd	Fumarate reductase, flavoprotein subunit	*lp_1113* (*lp_1113*, F9UMS2)	1.3.5.4	WCFS1	[41,42,285]
Fum	Fumarate dehydratase	*fum* (*lp_1112*, F9UMS1)	4.2.1.2	WCFS1	[41,42,285]
Ldh	L-Lactate dehydrogenase	*hicD1* (*lp_0350*, F9UTU9), *hicD2* (*lp_1245*, F9UN38), *hicD3* (*lp_2349*, F9UQQ1)	1.1.1.27	WCFS1	[41,42,285]
	D-Lactate dehydrogenase	*ldhD* (*lp_2057*, Q88VJ2)	1.1.1.28	WCFS1	[41,42,285]
Mae	Malate dehydrogenase (oxaloacetate-decarboxylating)	*mae* (*lp_1105*, F9UMR6)	1.1.1.38	WCFS1	[41,42,285]
Mdh	Malate dehydrogenase	*mdh* (LP80_117, not reported)	1.1.1.37	80	[37]
Mle	Malolactic enzyme	*mleS* (*lp_1118*, F9UMS6)	4.1.1.101	WCFS1	[41,42,285]
Pta	Phosphate acetyltransferase	*pta* (*lp_0807*, F9UM27)	2.3.1.8	WCFS1	[41,42,285]

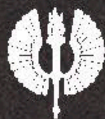
Pdh	Pyruvate dehydrogenase complex, E1, α-subunit	*pdhA* (*lp_2154*, F9UQ93)	1.2.4.1	WCFS1	[41,42,285]
	Pyruvate dehydrogenase complex, E1, β-subunit	*pdhB* (*lp_2153*, F9UQ92)	1.2.4.1	WCFS1	[41,42,285]
	Pyruvate dehydrogenase complex, E2	*pdhC* (*lp_2152*, F9UQ91)	2.3.1.12	WCFS1	[41,42,285]
	Pyruvate dehydrogenase complex, E3 component, dihydrolipoamide dehydrogenase	*pdhD* (*lp_2151*, F9UQ90)	1.8.1.4	WCFS1	[41,42,285]
Pfl	Formate acetyltransferase activating enzyme	*pflA* (*lp_3314*, F9UTJ6)	1.97.1.4	WCFS1	[41,42,285]
	Formate acetyltransferase	*pflB* (*lp_3313*, F9UTJ5)	2.3.1.54	WCFS1	[41,42,285]
Ttd	L(+)-Tartrate dehydratase, α-subunit	*ttdA* (*lp_1090*, F9UMQ6)	4.2.1.32	WCFS1	[41,42,285]
	L(+)-Tartrate dehydratase, β-subunit	*ttdB* (*lp_1089*, F9UMQ5)	4.2.1.32	WCFS1	[41,42,285]
Figure 4					
Dqd	3-Dehydroquinate dehydratase	*aroC1* (*lp_2798*, F9URR4), *aroC2* (*lp_3493*, Q88SD8)	4.2.1.10	WCFS1	[41,42,285]
Dsd	3-Dehydroshikimate dehydratase	Not reported	4.2.1.118	Not reported	[60]
Pad	Flavin prenyltransferase	*lpdB* (*lp_0271*, F9UT67)	2.5.1.129	WCFS1	[41,42,285]
Pad	Gallate decarboxylase (lpdC)	*lpdC* (*lp_2945*, F9US27)	4.1.1.59	WCFS1	[41,42,285]
Pad	Protein with unknown function (lpdD)	Not reported		WCFS1	[41,42,285]
Qsd	Shikimate/quinate 5-dehydrogenase	*aroD2* (A0A7J8KX38)	1.1.1.282	CMPG5300	[258]
Sd	Shikimate 5-dehydrogenase	*aroD1* (*lp_1084*, F9UMQ0), *aroD2* (*lp_3494*, F9UUG0), *aroD3* (*lp_3498*, F9UUG4), *aroD4* (*lp_3499*, F9UUG5)	1.1.1.25	WCFS1	[41,42,285]
Figure 8					
GacP	Cation transport protein	*lp_2943* (lp_2943, F9US26)		WCFS1	[41,42,285]
Lp_2739	ABC transporter ATP-binding protein	*lp_2739* (lp_2739, F9URL3)		WCFS1	[41,42,285]
Lp_2740	ABC transporter permease	*lp_2740* (lp_2740, F9URL4)		WCFS1	[41,42,285]
TanB	Tannase	*tanL* (lp_2956, F9US92)	3.1.1.20	WCFS1	[41,42,285]
TanR	LysR family transcriptional regulator	*lp_2942* (lp_2942, F9US25)			
Figure 9					
Hcr	NADPH-dependent FMN reductase	*lp_1424* (lp_1424, F9UNH2), also referred as *hcrA*	1.-.-.-	WCFS1	[41,42,285]
Hcr	NADPH-dependent FMN reductase	*lp_1425* (lp_1425, F9UNH3), also referred as *hcrB*	1.3.99.33	WCFS1	[41,42,285]

Lp_3629	β-Galactosidase	*bgl* (*lp_3629*, F9ULH8)	3.2.1.86	WCFS1	41,42,285
Pdc	Phenolic acid decarboxylase	*padA* (*lp_3665*, F9ULL2)	4.1.1.-	WCFS1	41,42,285
Pdc	Putative phenolic acid decarboxylase	Gene to be identified (add reference)			
VprA	Vinyl phenol reductase (annotated as "Fumarate reductase, flavoprotein subunit")	*lp_3125* (*lp_3125*, F9USN6)	1.3.99.1	WCFS1	41,42,285
α-Rhamn.	α-L-Rhamnosidase	*rhaB1* (not reported, C4PG47)	3.2.1.40	NCC245	258
	α-L-Rhamnosidase	*ram2* (*lp_3473*, F9UUE0)	3.2.1.40	WCFS1	41,42,285
	RhaB2	*rhaB2* (not reported, C4PG45)	3.2.1.40	NCC245	258
Figure 12					
ACK	Acetate kinase	*ack1* (*lp_0210*, F9UT17), *ack2* (*lp_0310*, F9UTR4), *ack3* (*lp_2242*, Q88V41)	2.7.2.1	WCFS1	41,42,285
AlcDH	1,3-Propanediol dehydrogenase	*dhaT* (*lp_3051*, F9USH3)	1.1.1.202	WCFS1	41,42,285
	Alcohol dehydrogenase	*adh1* (*lp_1665*, F9UP30), *adh2* (*lp_2873*, F9URX3)	1.1.1.1	WCFS1	41,42,285
	Bifunctional protein: alcohol dehydrogenase; acetaldehyde dehydrogenase	*adhE* (*lp_3662*, F9ULK9)	1.2.1.10 / 1.1.1.1	WCFS1	41,42,285
AldDH	Acetaldehyde dehydrogenase	*acdH* (*lp_0329*, F9UTT1)	1.2.1.10	WCFS1	41,42,285
	Bifunctional protein: alcohol dehydrogenase; acetaldehyde dehydrogenase	*adhE* (*lp_3662*, F9ULK9)	1.1.1.1 / 1.2.1.10	WCFS1	41,42,285
AraT	Aromatic amino acid aminotransferase	*araT1* (*lp_1280*, F9UN65), *araT2* (*lp_2684*, F9URG9)	2.6.1.-	WCFS1	41,42,285
BcAT	Branched-chain amino acid aminotransferase	*bcaT* (*lp_2390*, F9UQT2)	2.6.1.42	WCFS1	41,42,285
CBL / CGL	cystathionine β-lyase / cystathionine γ-lyase	*cblB* (*lp_0255*, F9UT53), *cblA1* (*lp_2751*, F9URM4), *clbA2* (*lp_2888*, F9URY4), *cblA3* (*lp_3517*, F9UUI1)	4.4.1.13	WCFS1	41,42,285
CBS	Cystathionine β-synthase	*cbs* (*lp_0256*, F9UT54)	2.5.1.47	WCFS1	41,42,285
CGS	Cysteine γ-synthase/O-succinylhomoserine (thiol)-lyase	*cgs* (*lp_2634*, F9URD2)	2.5.1.48	WCFS1	41,42,285
GDH	Glutamate dehydrogenase	*gdh* (*lp_1169*, F9UMW9)	1.4.1.4	WCFS1	41,42,285
KADH complex	Pyruvate dehydrogenase complex, E1, α-subunit	*pdhA* (*lp_2154*, F9UQ93)	1.2.4.1	WCFS1	41,42,285
	Pyruvate dehydrogenase complex, E1, β-subunit	*pdhB* (*lp_2153*, F9UQ92)	1.2.4.1	WCFS1	41,42,285

	Pyruvate dehydrogenase complex, E2	*pdhC* (*lp_2152*, F9UQ91)	2.3.1.12	WCFS1	[41,42,285]
	Pyruvate dehydrogenase complex, E3 component, dihydrolipoamide dehydrogenase	*pdhD* (*lp_2151*, F9UQ90)	1.8.1.4	WCFS1	[41,42,285]
KDC	Keto acid decarboxylase	No gene identified			
LDH	L-Lactate dehydrogenase	*hicD1* (*lp_0350*, F9UTU9), *hicD2* (*lp_1245*, F9UN38), *hicD3* (*lp_2349*, F9UQQ1)	1.1.1.27	WCFS1	[41,42,285]
MAT	L-Glutamine-4-(methylsulfanyl)-2-oxobutanoate aminotransferase	*dapC* (*lp_0339*, F9UTU1)	2.6.1.117	WCFS1	[41,42,285]
PTA	Phosphotransacylase	*eutD* (not reported, T5JSR8)		EGD-AQ4	[258]
Figure 13					
ADI	Arginine deiminase	*arcA* (not reported, Q6U3A0)	3.5.3.6	N.r.	[258]
CK	Carbamate kinase	*arcC* (not reported, B8XRB1)	2.7.2.2	N.r.	[258]
HDC	Histidine decarboxylase	*hdc* (not reported)		N.r.	[135]
LDC	Arginine/lysine/ornithine decarboxylase	*SN13T_1490* (not reported, A0A7H4UGA5)		N.r.	[258]
NLE	Multicopper oxidase	*sufI* (not reported, D7V8D2)	1.10.3.2	ATCC 14917	[258]
	Multicopper oxidase	*lp_0355* (*lp_0355*, F9UTV3)		WCFS1	[41,42,285]
ODC	Arginine/lysine/ornithine decarboxylase	*SN13T_1490* (not reported, A0A7H4UGA5)		N.r.	[258]
OTC	Ornithine transcarbamoyltransferase	*arcB* (not reported, A0A387DQY2)	2.1.3.3	N.r.	[258]
TDC	Tyrosine decarboxylase	*tdc* (not reported, A2I9X8)	4.1.1.25	N.r.	[258]

Supplementary Table S2. Full names f the sea buckthorn cultivars and biotypes abbreviated in the **Figure 18** in addition to growth location and subspecies.

Short name	***Full name***	***Location***	***Subspecies***	***Ref.***
AVG	Avgustinka	Finland	*mongolica*	[268]
BOT	Botanicheskaya	Finland	*mongolica*	[268]
CHU	Chuiskaya	Finland	*mongolica*	[268]
ORA	Oranzhevaya	Finland	*mongolica*	[268]
PRE	Prevoshodnaya	Finland	*mongolica*	[268]
RAI	Raisa	Finland	*rhamnoides*	[268]
TRO	Trofimovskaya	Finland	*mongolica*	[268]
AU	Auraş	Romania	*carpatica*	[267]
B6AU	Auraş yellow biotype round fruit	Romania	*carpatica*	[267]
B6SER	Serpenta biotype-elongate fruit	Romania	*carpatica*	[267]
B6VIC	Victoria biotype fruit	Romania	*carpatica*	[267]
BS1	Biotype Ştiulete 1	Romania	*carpatica*	[267]
BS2	Biotype Ştiulete 2	Romania	*carpatica*	[267]
OV	Ovidiu	Romania	*carpatica*	[267]
RF	Red fruits	Romania	*carpatica*	[267]
SER	Serpenta	Romania	*carpatica*	[267]
SIL	Silvia	Romania	*carpatica*	[267]
TIB	Tiberiu	Romania	*carpatica*	[267]
VIC	Victoria	Romania	*carpatica*	[267]



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